



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<p>(21) International Application Number: PCT/US99/28588</p> <p>(22) International Filing Date: 2 December 1999 (02.12.99)</p> <p>(30) Priority Data: 60/110,781 3 December 1998 (03.12.98) US</p> <p>(71) Applicant (for all designated States except US): E.I. DU PONT DE NEMOURS AND COMPANY [US/US]; 1007 Market Street, Wilmington, DE 19898 (US).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only): CAHOON, Rebecca, E. [US/US]; 2331 West 18th Street, Wilmington, DE 19806 (US). COUGHLAN, Sean, J. [US/US]; 108 Stratton Drive, Hockessin, DE 19707 (US). MIAO, Guo-Hua [US/US]; 202 Cherry Blossom Place, Hockessin, DE 19707 (US). RAFALSKI, J., Antoni [US/US]; 2028 Longcome Drive, Wilmington, DE 19810 (US).</p> <p>(74) Agent: FEULNER, Gregory, J.; E.I. du Pont de Nemours and Company, Legal Patent Records Center, 1007 Market Street, Wilmington, DE 19898 (US).</p>		<p>(81) Designated States: AE, AL, AU, BA, BB, BG, BR, CA, CN, CR, CU, CZ, DM, EE, GD, GE, HR, HU, ID, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, UA, US, UZ, VN, YU, ZA, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p><b>Published</b> Without international search report and to be republished upon receipt of that report.</p>
<p>(54) Title: PLANT VITAMIN E BIOSYNTHETIC ENZYMES</p> <p>(57) Abstract</p> <p>This invention relates to an isolated nucleic acid fragment encoding a vitamin E biosynthetic enzyme. The invention also relates to the construction of a chimeric gene encoding all or a portion of the vitamin E biosynthetic enzyme, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of the vitamin E biosynthetic enzyme in a transformed host cell.</p> <pre>SEQ ID NO:39  HVYHVPKHAL-----FLATCYFS----- SEQ ID NO:40  KKATLAAPSLTSLP-----VTKNSS-FGKSSLLFRSPSSSSVS-MTTRGHV SEQ ID NO:38  NNN-----SKRPA-PLTFLHRLDAAPPPRPSLGHANRPPRPLVLLPAR-KLRAPOGV SEQ ID NO:28  HATVVR-----PTISCHHTFSGSPRTFARIRVGPASVPIRASA-ASERGEI SEQ ID NO:08  HATVVR-----PTISCHHTFSGSPRTFARIRVGPASVPIRASA-ASERGEI SEQ ID NO:22  NANAALLHCSSSR-SLACRGRSHYRAPH-VPRSRRLRAVSVL-----R-PHASTA- SEQ ID NO:24  AR-VQPTGALAPHLRLCTSRHLICASAPRAGLCLNRRRRRRSSRRKTLAVRANAP SEQ ID NO:26  FNN-----GH-----AL-----60  1 SEQ ID NO:39  LTHASATIASADLYEKIKMYFDSGLMEDYNGDHNGHYGPGCTYRI-----ORRQDI SEQ ID NO:40  AVAAATSTEA-LANGIAEFTVYSGLCIEYNGDHNGHYGDSGLVSLDSGHHKAOI SEQ ID NO:38  VDDRGPGDAAPFGLKEGAGLYDESSGLMESINGEHNGHYGDSGEAASHSD--HRAAOI SEQ ID NO:28  VLEQKPKKDDKKLQNGIAEYFDESSGLMENMGDHNGHYGDSSTVLSLSD--HRAAOI SEQ ID NO:08  VLEQKPKKDDKKLQNGIAEYFDESSGLMENMGDHNGHYGDSSTVLSLSD--HRAAOI SEQ ID NO:22  ---QAPATAPGLKEGAGLYDESSGLMENMGDHNGHYGDSGEAASHSD--HRAAOI SEQ ID NO:24  LSSSTAAANAPFGLKEGAGLYDESSGLMESINGEHNGHYGDSGEAASHSD--HRAAOI SEQ ID NO:26  -----120  61 SEQ ID NO:39  DLIKELLAMAVPNSA-----KPKILDLGGGIGSSLYLAQOHAQVHGASLSFVQVRA SEQ ID NO:40  RMIEESLRFAGVTDEE-EKKIKKVVVGGGIGSSRYLASKFGAECTGITLSFVQAKRA SEQ ID NO:38  RMIEELAFAAV-----DDPTNRFTTIVDGGGIGSSRYLANKYGAQSGITLSFVQARG SEQ ID NO:28  RMIEESLRFASV-----SEERKPKFSTIVDGGGIGSSRYLAKFGATSGVITLSFVQARG SEQ ID NO:08  RMIEELAFASV-----SEERKPKFSTIVDGGGIGSSRYLAKFGATSGVITLSFVQARG SEQ ID NO:22  RMIEELAFAGVPSDDPKPTKTIVDGGGIGSSRYLANKYGAQSGITLSFVQARG SEQ ID NO:24  RMIEELAF-----240 SEQ ID NO:26  -----180  121 SEQ ID NO:39  GERARALGSGTCQFOVANAALDPPASDTFMVNSLESGEHPKQAFLOEAMVRLKPGG SEQ ID NO:40  MDLAAQGLSKRASFOVADALDQPPGDFDLVNSHESGERHPDKAFVGLRVAAAPGG SEQ ID NO:38  MALAAQGLSKRASFOVADALDQPPGDFDLVNSHESGERHPDKAFVGLRVAAAPGG SEQ ID NO:28  MALAAQGLSKRASFOVADALDQPPGDFDLVNSHESGERHPDKAFVGLRVAAAPGG SEQ ID NO:08  MALAAQGLSKRASFOVADALDQPPGDFDLVNSHESGERHPDKAFVGLRVAAAPGG SEQ ID NO:22  MALAAQGLSDQVTLQVADALDQPPGDFDLVNSHESGERHPDKAFVGLRVAAAPGG SEQ ID NO:24  -----240 SEQ ID NO:26  -----240  181 SEQ ID NO:39  RLIIATWCHNRDIDMGGLYADERRHLOAIYDVCYLPYVSLPDYEATACGFGIHTA SEQ ID NO:40  RLIIATWCHNRHLSAGEALQWQONLIDKIKTFYLPANCSTDDYVNLQSHSLDITKA SEQ ID NO:38  RLIIATWCHNRHLSAGEALQWQONLIDKIKTFYLPANCSTDDYVNLQSHSLDITKA SEQ ID NO:28  RLIIATWCHNRHLSAGEALQWQONLIDKIKTFYLPANCSTDDYVNLQSHSLDITKA SEQ ID NO:08  RLIIATWCHNRHLSAGEALQWQONLIDKIKTFYLPANCSTDDYVNLQSHSLDITKA SEQ ID NO:22  RLIIATWCHNRHLSAGEALQWQONLIDKIKTFYLPANCSTDDYVNLQSHSLDITKA SEQ ID NO:24  RLIIATWCHNRHLSAGEALQWQONLIDKIKTFYLPANCSTDDYVNLQSHSLDITKA SEQ ID NO:26  RLIIATWCHNRHLSAGEALQWQONLIDKIKTFYLPANCSTDDYVNLQSHSLDITKA300  241 SEQ ID NO:39  DMSVAPFVDRVIESAFDPRVIMAGQAGFKIHAALCLRLKMGYRGVLRFOLLTGI SEQ ID NO:40  DMSVAPFVPAVIRALTWKGWLSLRSGHRSIKGALTPLMIEGTGKGVKFGIITCO SEQ ID NO:38  DMSVAPFVPAVIRALTWKGWLSLRSGHRSIKGALTPLMIEGTGKGVKFGIITCO SEQ ID NO:28  DMSVAPFVPAVIRALTWKGWLSLRSGHRSIKGALTPLMIEGTGKGVKFGIITCO SEQ ID NO:08  DMSVAPFVPAVIRALTWKGWLSLRSGHRSIKGALTPLMIEGTGKGVKFGIITCO SEQ ID NO:22  DMSVAPFVPAVIRALTWKGWLSLRSGHRSIKGALTPLMIEGTGKGVKFGIITCO SEQ ID NO:24  DMSVAPFVPAVIRALTWKGWLSLRSGHRSIKGALTPLMIEGTGKGVKFGIITCO SEQ ID NO:26  DMSVAPFVPAVIRALTWKGWLSLRSGHRSIKGALTPLMIEGTGKGVKFGIITCO360  301 SEQ ID NO:39  KPLV----- SEQ ID NO:40  KPLV----- SEQ ID NO:38  KPNALIEGEPEASPSVE.- SEQ ID NO:28  K-----PE.- SEQ ID NO:08  SYVDFYFHTA.- SEQ ID NO:22  KPCA--NA-----HALLK SEQ ID NO:24  -----Q.- SEQ ID NO:26  KPETT-----380  361</pre>		

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TITLE

## PLANT VITAMIN E BIOSYNTHETIC ENZYMES

This application claims the benefit of U.S. Provisional Application No. 60/110,781, filed December 3, 1998.

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FIELD OF THE INVENTION

This invention is in the field of plant molecular biology. More specifically, this invention pertains to nucleic acid fragments encoding vitamin E biosynthetic enzymes in plants and seeds.

BACKGROUND OF THE INVENTION

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Vitamin E (alpha-tocopherol) is an essential element in the mammalian diet since mammals can not synthesize plastoquinones or tocopherols. The first step in the formation of plastoquinones and tocopherols in plants is the formation of homogениstate from 4-hydroxyphenylpyruvate, a reaction catalyzed by 4-hydroxyphenylpyruvate dioxygenase (EC 1.13.11.27). Genetic mutants of this enzyme in *Arabidopsis* are deficient in both carotenoids and tocopherols (Norris et al. (1995) *Plant Cell* 7:2139-2149). Carotenoids (xanthopylls) in corn endosperm are valuable coloring agents in poultry feeds; tocopherols are antioxidants which may be important in oil stability and human health. Generally present at low levels in plant tissues, 4-hydroxyphenylpyruvate dioxygenase catalyzes a complex, irreversible reaction. Only recently has 4-hydroxyphenylpyruvate dioxygenase been purified to homogeneity from a plant source (Garcia et al. (1997 *Biochem. J.* 325:761-769). In plants, cDNAs encoding 4-hydroxyphenylpyruvate dioxygenase have been identified in carrots, barley and *Arabidopsis thaliana* with at least two different variants existing in this last plant. 4-Hydroxyphenylpyruvate dioxygenase is a known herbicide target (Mayonado et al. (1989) *Pestic. Biochem. Physiol.* 35:138-145; Schultz et al. (1993) *FEBS lett.* 318:162-166; Secor (1994) *Plant Phys.* 106:1429-1433). Even though plastoquinones and tocopherols are not synthesized by mammals and bacteria 4-hydroxyphenylpyruvate dioxygenase activity is present, often at high levels, and involved in phenylalanine and tyrosine degradation. Among others, the cDNAs encoding 4-hydroxyphenylpyruvate dioxygenase have been identified in *Mycosphaerella graminicola*, mice and *Coccidioides immitis*.

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Plants synthesize alpha, beta, gamma and delta tocopherols of which alpha tocopherol (vitamin E) has the highest value for human nutrition. In soybean 7% of the tocopherols are vitamin E. Gamma tocopherol methyltransferase catalyzes the final step in vitamin E synthesis and has been purified to homogeneity from pepper, marigold, *Euglena* and spinach. The gene encoding gamma tocopherol methyltransferase from *Synechocystis* was identified by mutating an open reading frame encoding a methyltransferase located in the operon containing the 4-hydroxyphenylpyruvate dioxygenase gene in the *Synechocystis*

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genomic sequence (Shintani and Della Penna (1998) Abstract for the American Society of Plant Physiologists meeting in Madison, WI).

Since mammals can not synthesize tocopherols, the enzymes described here may be used for the discovery of new herbicides.

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#### SUMMARY OF THE INVENTION

The present invention relates to isolated polynucleotides comprising a nucleotide sequence encoding a polypeptide of at least 160 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of a corn gamma-tocopherol methyltransferase polypeptide of SEQ ID  
10 NOs:2 and 22, a rice gamma-tocopherol methyltransferase polypeptide of SEQ ID NOs:4, 6, 24, and 26, a soybean gamma-tocopherol methyltransferase polypeptide of SEQ ID NOs:8 and 28, and a wheat gamma-tocopherol methyltransferase polypeptide of SEQ ID NOs:10 and 30. The present invention also relates to an isolated polynucleotide comprising the complement of the nucleotide sequences described above.

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The present invention relates to isolated polynucleotides comprising a nucleotide sequence encoding a polypeptide of at least 150 amino acids that has at least 95% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of a catalpa 4-hydroxyphenylpyruvate dioxygenase polypeptide of SEQ ID  
20 NO:32, a rice 4-hydroxyphenylpyruvate dioxygenase polypeptide of SEQ ID NOs:12, 14, and 34, a soybean 4-hydroxyphenylpyruvate dioxygenase polypeptide of SEQ ID NOs:16 and 36, a Vernonia 4-hydroxyphenylpyruvate dioxygenase of SEQ ID NO:18, and a wheat 4-hydroxyphenylpyruvate dioxygenase polypeptide of SEQ ID NOs:20 and 38. The present invention also relates to an isolated polynucleotide comprising the complement of the nucleotide sequences described above.

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It is preferred that the isolated polynucleotides of the claimed invention consist of a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, and 37 that codes for the polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, and 38. The present invention also relates to an isolated polynucleotide  
30 comprising a nucleotide sequences of at least one of 60 (preferably at least one of 40, most preferably at least one of 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, and the complement of such nucleotide sequences.

The present invention relates to a chimeric gene comprising an isolated polynucleotide  
35 of the present invention operably linked to suitable regulatory sequences.

The present invention relates to an isolated host cell comprising a chimeric gene of the present invention or an isolated polynucleotide of the present invention. The host cell may be eukaryotic, such as a yeast or a plant cell, or prokaryotic, such as a bacterial cell. The

present invention also relates to a virus, preferably a baculovirus, comprising an isolated polynucleotide of the present invention or a chimeric gene of the present invention.

5 The present invention relates to a process for producing an isolated host cell comprising a chimeric gene of the present invention or an isolated polynucleotide of the present invention, the process comprising either transforming or transfecting an isolated compatible host cell with a chimeric gene or isolated polynucleotide of the present invention.

10 The present invention relates to a gamma-tocopherol methyltransferase polypeptide of at least 160 amino acids comprising at least 80% homology based on the Clustal method of alignment compared to a polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 22, 24, 26, 28, and 30.

The present invention relates to a 4-hydroxyphenylpyruvate dioxygenase polypeptide of at least 150 amino acids comprising at least 95% homology based on the Clustal method of alignment compared to a polypeptide selected from the group consisting of SEQ ID NOs:12, 14, 16, 18, 20, 32, 34, 36, and 38.

15 The present invention relates to a method of selecting an isolated polynucleotide that affects the level of expression of a gamma-tocopherol methyltransferase or a 4-hydroxyphenylpyruvate dioxygenase polypeptide in a host cell, preferably a plant cell, the method comprising the steps of: (a) constructing an isolated polynucleotide of the present invention or an isolated chimeric gene of the present invention; (b) introducing the isolated  
20 polynucleotide or the isolated chimeric gene into a host cell; (c) measuring the level a gamma-tocopherol methyltransferase or a 4-hydroxyphenylpyruvate dioxygenase polypeptide in the host cell containing the isolated polynucleotide; and (d) comparing the level of a gamma-tocopherol methyltransferase or a 4-hydroxyphenylpyruvate dioxygenase polypeptide in the host cell containing the isolated polynucleotide with the level of a gamma-  
25 tocopherol methyltransferase or a 4-hydroxyphenylpyruvate dioxygenase polypeptide in the host cell that does not contain the isolated polynucleotide.

The present invention relates to a method of obtaining a nucleic acid fragment encoding a substantial portion of a gamma-tocopherol methyltransferase or a 4-hydroxyphenylpyruvate dioxygenase polypeptide gene, preferably a plant gamma-tocopherol  
30 methyltransferase or 4-hydroxyphenylpyruvate dioxygenase polypeptide gene, comprising the steps of : synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least one of 60 (preferably at least one of 40, most preferably at least one of 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, and the  
35 complement of such nucleotide sequences; and amplifying a nucleic acid fragment (preferably a cDNA inserted in a cloning vector) using the oligonucleotide primer. The amplified nucleic acid fragment preferably will encode a portion of a gamma-tocopherol methyltransferase or a 4-hydroxyphenylpyruvate dioxygenase amino acid sequence.

The present invention also relates to a method of obtaining a nucleic acid fragment encoding all or a substantial portion of the amino acid sequence encoding a 4-hydroxyphenylpyruvate dioxygenase or a gamma tocopherol methyltransferase polypeptide comprising the steps of: probing a cDNA or genomic library with an isolated polynucleotide of the present invention; identifying a DNA clone that hybridizes with an isolated polynucleotide of the present invention; isolating the identified DNA clone; and sequencing the cDNA or genomic fragment that comprises the isolated DNA clone.

A further embodiment of the instant invention is a method for evaluating at least one compound for its ability to inhibit the activity of a 4-hydroxyphenylpyruvate dioxygenase or a gamma tocopherol methyltransferase, the method comprising the steps of:

- (a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding a 4-hydroxyphenylpyruvate dioxygenase or a gamma tocopherol methyltransferase, operably linked to suitable regulatory sequences;
- (b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of 4-hydroxyphenylpyruvate dioxygenase or gamma tocopherol methyltransferase in the transformed host cell;
- (c) optionally purifying the 4-hydroxyphenylpyruvate dioxygenase or the gamma tocopherol methyltransferase expressed by the transformed host cell;
- (d) treating the 4-hydroxyphenylpyruvate dioxygenase or the gamma tocopherol methyltransferase with a compound to be tested; and
- (e) comparing the activity of the 4-hydroxyphenylpyruvate dioxygenase or the gamma tocopherol methyltransferase that has been treated with a test compound to the activity of an untreated 4-hydroxyphenylpyruvate dioxygenase or gamma tocopherol methyltransferase, thereby selecting compounds with potential for inhibitory activity.

The present invention relates to a composition, such as a hybridization mixture, comprising an isolated polynucleotide of the present invention.

The present invention relates to an isolated polynucleotide of the present invention comprising at least one of 30 contiguous nucleotides derived from a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, and 37.

The present invention relates to an expression cassette comprising an isolated polynucleotide of the present invention operably linked to a promoter.

The present invention relates to a method for positive selection of a transformed cell comprising: (a) transforming a host cell with the chimeric gene of the present invention or an expression cassette of the present invention; and (b) growing the transformed host cell, preferably plant cell, such as a monocot or a dicot, under conditions which allow expression of the gamma-tocopherol methyltransferase or a 4-hydroxyphenylpyruvate dioxygenase polynucleotide in an amount sufficient to complement a null mutant to provide a positive selection means.

BRIEF DESCRIPTION OF THE  
DRAWINGS AND SEQUENCE DESCRIPTIONS

The invention can be more fully understood from the following detailed description and the accompanying drawings and Sequence Listing which form a part of this application.

5        Figure 1 shows a comparison of the amino acid sequences of the gamma-tocopherol methyltransferase from soybean contig assembled from clones sah1c.pk001.k8, sgc5c.pk001.m23, and sah1c.pk004.g2 (SEQ ID NO:8), corn contig assembled from clones cr1n.pk0179.f10:fis, cs1.pk0065.f2, cta1n.pk0031.d2, p0060.coran49r, and p0103.ciaay86r (SEQ ID NO:22, the 3'-end sequence of rice clone rls72.pk0010.g3 (SEQ ID NO:24), the 5'-  
10      end sequence of clone rls72.pk0010.g3 (SEQ ID NO:26), soybean clone sah1c.pk004.g2 (SEQ ID NO:28), wheat clone wr1.pk0077.f1:fis (SEQ ID NO:30), *Synechocystis sp.* (NCBI General Identifier No. 1001725; SEQ ID NO:39), and *Arabidopsis thaliana* (NCBI General Identifier No. 4106538; SEQ ID NO:40). Dashes are used by the program to maximize the alignment.

15        Figure 2 shows a comparison of the amino acid sequences of the 4-hydroxyphenyl-pyruvate dioxygenase from catalpa clone ncs.pk0012.g1:fis (SEQ ID NO:32), soybean clone sgc5c.pk001.j9:fis (SEQ ID NO:36), wheat clone wdk4c.pk006.m9:fis (SEQ ID NO:38), *Hordeum vulgare* having NCBI General Identifier No. 3334222 (SEQ ID NO:41), *Daucus carota* having NCBI General Identifier No. 3334219 (SEQ ID NO:42), and *Arabidopsis*  
20      *thaliana* having NCBI General Identifier No. 3334223 (SEQ ID NO:43). Dashes are used by the program to maximize the alignment.

Figure 3 depicts the distribution of the percent of alpha- and gamma-tocopherol content of 25 transgenic soybean lines transformed with the gamma tocopherol methyltransferase sequence from clone sah1c.pk001.k8.

25        Table 1 lists the polypeptides that are described herein, the designation of the cDNA clones that comprise the nucleic acid fragments encoding polypeptides representing all or a substantial portion of these polypeptides, and the corresponding identifier (SEQ ID NO:) as used in the attached Sequence Listing. The sequence descriptions and Sequence Listing attached hereto comply with the rules governing nucleotide and/or amino acid sequence  
30      disclosures in patent applications as set forth in 37 C.F.R. §1.821-1.825.

**TABLE 1**  
**Vitamin E Biosynthetic Enzymes**

Protein	Clone Designation	SEQ ID NO:	
		(Nucleotide)	(Amino Acid)
Corn Gamma Tocopherol Methyltransferase	Contig of: cr1n.pk0179.f10 cs1.pk0065.e1 cs1.pk0065.f2 cta1n.pk0031.d2	1	2
Rice Gamma Tocopherol Methyltransferase	rl0n.pk085.e11	3	4
Rice Gamma Tocopherol Methyltransferase	Contig of: rl0n.pk099.d24 rls72.pk0010.g3	5	6
Soybean Gamma Tocopherol Methyltransferase	Contig of: sah1c.pk001.k8 sgc5c.pk001.m23 sah1c.pk004.g2	7	8
Wheat Gamma Tocopherol Methyltransferase	Contig of: wr1.pk0077.f1 wr1.pk177.b11 wle1n.pk0065.h9 wre1n.pk0111.d6	9	10
Rice 4-Hydroxyphenylpyruvate Dioxygenase	rlr12.pk0019.g5	11	12
Rice 4-Hydroxyphenylpyruvate Dioxygenase	rlr12.pk0025.e4	13	14
Soybean 4-Hydroxyphenylpyruvate Dioxygenase	Contig of: sgc5c.pk001.j9 sgs1c.pk002.a8 sfl1.pk126.n15 sgs1c.pk003.o5 sgc4c.pk001.p2	15	16
Vernonia 4-Hydroxyphenylpyruvate Dioxygenase	vs1.pk0015.b2	17	18
Wheat 4-Hydroxyphenylpyruvate Dioxygenase	Contig of: wdk4c.pk006.m9 wlmk1.pk0021.h10 wlmk1.pk0019.e2 wlm0.pk0035.d5 wlmk1.pk0013.g11	19	20
Corn Gamma Tocopherol Methyltransferase	Contig of: cr1n.pk0179.f10:fis cs1.pk0065.f2 cta1n.pk0031.d2 p0060.coran49r p0103.ciaay86r	21	22
Rice Gamma Tocopherol Methyltransferase	rls72.pk0010.g3-3'	23	24

Protein	Clone Designation	SEQ ID NO:	
		(Nucleotide)	(Amino Acid)
Rice Gamma Tocopherol Methyltransferase	rls72.pk0010.g3-5'	25	26
Soybean Gamma Tocopherol Methyltransferase	sah1c.pk004.g2	27	28
Wheat Gamma Tocopherol Methyltransferase	wr1.pk0077.f1:fis	29	30
Catalpa 4-Hydroxyphenyl-pyruvate Dioxygenase	ncs.pk0012.g1:fis	31	32
Rice 4-Hydroxyphenylpyruvate Dioxygenase	rlr12.pk0025.e4:fis	33	34
Soybean 4-Hydroxyphenyl-pyruvate Dioxygenase	sgc5c.pk001.j9:fis	35	36
Wheat 4-Hydroxyphenylpyruvate Dioxygenase	wdk4c.pk006.m9:fis	37	38

The Sequence Listing contains the one letter code for nucleotide sequence characters and the three letter codes for amino acids as defined in conformity with the IUPAC-IUBMB standards described in *Nucleic Acids Res.* 13:3021-3030 (1985) and in the *Biochemical J.* 219 (No. 2):345-373 (1984) which are herein incorporated by reference. The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. §1.822.

#### DETAILED DESCRIPTION OF THE INVENTION

In the context of this disclosure, a number of terms shall be utilized. As used herein, a "polynucleotide" is a nucleotide sequence such as a nucleic acid fragment. A polynucleotide may be a polymer of RNA or DNA that is single- or double-stranded, that optionally contains synthetic, non-natural or altered nucleotide bases. A polynucleotide in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA, synthetic DNA, or mixtures thereof. An isolated polynucleotide of the present invention may include at least one of 60 contiguous nucleotides, preferably at least one of 40 contiguous nucleotides, most preferably one of at least 30 contiguous nucleotides derived from SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, or the complement of such sequences.

As used herein, "contig" refers to a nucleotide sequence that is assembled from two or more constituent nucleotide sequences that share common or overlapping regions of sequence homology. For example, the nucleotide sequences of two or more nucleic acid fragments can be compared and aligned in order to identify common or overlapping sequences. Where common or overlapping sequences exist between two or more nucleic

acid fragments, the sequences (and thus their corresponding nucleic acid fragments) can be assembled into a single contiguous nucleotide sequence.

As used herein, "substantially similar" refers to nucleic acid fragments wherein changes in one or more nucleotide bases results in substitution of one or more amino acids, but do not affect the functional properties of the polypeptide encoded by the nucleotide sequence. "Substantially similar" also refers to nucleic acid fragments wherein changes in one or more nucleotide bases does not affect the ability of the nucleic acid fragment to mediate alteration of gene expression by gene silencing through for example antisense or co-suppression technology. "Substantially similar" also refers to modifications of the nucleic acid fragments of the instant invention such as deletion or insertion of one or more nucleotides that do not substantially affect the functional properties of the resulting transcript vis-à-vis the ability to mediate gene silencing or alteration of the functional properties of the resulting protein molecule. It is therefore understood that the invention encompasses more than the specific exemplary nucleotide or amino acid sequences and includes functional equivalents thereof.

Substantially similar nucleic acid fragments may be selected by screening nucleic acid fragments representing subfragments or modifications of the nucleic acid fragments of the instant invention, wherein one or more nucleotides are substituted, deleted and/or inserted, for their ability to affect the level of the polypeptide encoded by the unmodified nucleic acid fragment in a plant or plant cell. For example, a substantially similar nucleic acid fragment representing at least one of 30 contiguous nucleotides derived from the instant nucleic acid fragment can be constructed and introduced into a plant or plant cell. The level of the polypeptide encoded by the unmodified nucleic acid fragment present in a plant or plant cell exposed to the substantially similar nucleic fragment can then be compared to the level of the polypeptide in a plant or plant cell that is not exposed to the substantially similar nucleic acid fragment.

For example, it is well known in the art that antisense suppression and co-suppression of gene expression may be accomplished using nucleic acid fragments representing less than the entire coding region of a gene, and by nucleic acid fragments that do not share 100% sequence identity with the gene to be suppressed. Moreover, alterations in a nucleic acid fragment which result in the production of a chemically equivalent amino acid at a given site, but do not effect the functional properties of the encoded polypeptide, are well known in the art. Thus, a codon for the amino acid alanine, a hydrophobic amino acid, may be substituted by a codon encoding another less hydrophobic residue, such as glycine, or a more hydrophobic residue, such as valine, leucine, or isoleucine. Similarly, changes which result in substitution of one negatively charged residue for another, such as aspartic acid for glutamic acid, or one positively charged residue for another, such as lysine for arginine, can also be expected to produce a functionally equivalent product. Nucleotide changes which

result in alteration of the N-terminal and C-terminal portions of the polypeptide molecule would also not be expected to alter the activity of the polypeptide. Each of the proposed modifications is well within the routine skill in the art, as is determination of retention of biological activity of the encoded products. Consequently, an isolated polynucleotide comprising a nucleotide sequence of at least one of 60 (preferably at least one of 40, most preferably at least one of 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and the complement of such nucleotide sequences may be used in methods of selecting an isolated polynucleotide that affects the expression of a polypeptide (vitamin E biosynthetic enzyme) in a host cell. A method of selecting an isolated polynucleotide that affects the level of expression of a polypeptide in a host cell (eukaryotic, such as plant or yeast, prokaryotic such as bacterial, or viral) may comprise the steps of: constructing an isolated polynucleotide of the present invention or an isolated chimeric gene of the present invention; introducing the isolated polynucleotide or the isolated chimeric gene into a host cell; measuring the level a polypeptide in the host cell containing the isolated polynucleotide; and comparing the level of a polypeptide in the host cell containing the isolated polynucleotide with the level of a polypeptide in a host cell that does not contain the isolated polynucleotide.

Moreover, substantially similar nucleic acid fragments may also be characterized by their ability to hybridize. Estimates of such homology are provided by either DNA-DNA or DNA-RNA hybridization under conditions of stringency as is well understood by those skilled in the art (Hames and Higgins, Eds. (1985) Nucleic Acid Hybridisation, IRL Press, Oxford, U.K.). Stringency conditions can be adjusted to screen for moderately similar fragments, such as homologous sequences from distantly related organisms, to highly similar fragments, such as genes that duplicate functional enzymes from closely related organisms. Post-hybridization washes determine stringency conditions. One set of preferred conditions uses a series of washes starting with 6X SSC, 0.5% SDS at room temperature for 15 min, then repeated with 2X SSC, 0.5% SDS at 45°C for 30 min, and then repeated twice with 0.2X SSC, 0.5% SDS at 50°C for 30 min. A more preferred set of stringent conditions uses higher temperatures in which the washes are identical to those above except for the temperature of the final two 30 min washes in 0.2X SSC, 0.5% SDS was increased to 60°C. Another preferred set of highly stringent conditions uses two final washes in 0.1X SSC, 0.1% SDS at 65°C.

Substantially similar nucleic acid fragments of the instant invention may also be characterized by the percent identity of the amino acid sequences that they encode to the amino acid sequences disclosed herein, as determined by algorithms commonly employed by those skilled in this art. Suitable nucleic acid fragments (isolated polynucleotides of the present invention) encode polypeptides that are at least about 70% identical, preferably at

least about 80% identical to the amino acid sequences reported herein. Preferred nucleic acid fragments encode amino acid sequences that are at least about 85% identical to the amino acid sequences reported herein. More preferred nucleic acid fragments encode amino acid sequences that are at least about 90% identical to the amino acid sequences reported herein. Most preferred are nucleic acid fragments that encode amino acid sequences that are at least about 95% identical to the amino acid sequences reported herein. Suitable nucleic acid fragments not only have the above homologies but typically encode a polypeptide having at least about 50 amino acids, preferably at least about 100 amino acids, more preferably at least about 150 amino acids, still more preferably at least about 200 amino acids, and most preferably at least about 250 amino acids. Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS* 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5.

A "substantial portion" of an amino acid or nucleotide sequence comprises an amino acid or a nucleotide sequence that is sufficient to afford putative identification of the protein or gene that the amino acid or nucleotide sequence comprises. Amino acid and nucleotide sequences can be evaluated either manually by one skilled in the art, or by using computer-based sequence comparison and identification tools that employ algorithms such as BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol.* 215:403-410; see also [www.ncbi.nlm.nih.gov/BLAST/](http://www.ncbi.nlm.nih.gov/BLAST/)). In general, a sequence of ten or more contiguous amino acids or thirty or more contiguous nucleotides is necessary in order to putatively identify a polypeptide or nucleic acid sequence as homologous to a known protein or gene. Moreover, with respect to nucleotide sequences, gene-specific oligonucleotide probes comprising 30 or more contiguous nucleotides may be used in sequence-dependent methods of gene identification (e.g., Southern hybridization) and isolation (e.g., *in situ* hybridization of bacterial colonies or bacteriophage plaques). In addition, short oligonucleotides of 12 or more nucleotides may be used as amplification primers in PCR in order to obtain a particular nucleic acid fragment comprising the primers. Accordingly, a "substantial portion" of a nucleotide sequence comprises a nucleotide sequence that will afford specific identification and/or isolation of a nucleic acid fragment comprising the sequence. The instant specification teaches amino acid and nucleotide sequences encoding polypeptides that comprise one or more particular plant proteins. The skilled artisan, having the benefit of the sequences as reported herein, may now use all or a substantial portion of the disclosed sequences for purposes known to those skilled in this art. Accordingly, the instant invention

comprises the complete sequences as reported in the accompanying Sequence Listing, as well as substantial portions of those sequences as defined above.

“Codon degeneracy” refers to divergence in the genetic code permitting variation of the nucleotide sequence without effecting the amino acid sequence of an encoded polypeptide. Accordingly, the instant invention relates to any nucleic acid fragment comprising a nucleotide sequence that encodes all or a substantial portion of the amino acid sequences set forth herein. The skilled artisan is well aware of the “codon-bias” exhibited by a specific host cell in usage of nucleotide codons to specify a given amino acid. Therefore, when synthesizing a nucleic acid fragment for improved expression in a host cell, it is desirable to design the nucleic acid fragment such that its frequency of codon usage approaches the frequency of preferred codon usage of the host cell.

“Synthetic nucleic acid fragments” can be assembled from oligonucleotide building blocks that are chemically synthesized using procedures known to those skilled in the art. These building blocks are ligated and annealed to form larger nucleic acid fragments which may then be enzymatically assembled to construct the entire desired nucleic acid fragment. “Chemically synthesized”, as related to nucleic acid fragment, means that the component nucleotides were assembled *in vitro*. Manual chemical synthesis of nucleic acid fragments may be accomplished using well established procedures, or automated chemical synthesis can be performed using one of a number of commercially available machines. Accordingly, the nucleic acid fragments can be tailored for optimal gene expression based on optimization of nucleotide sequence to reflect the codon bias of the host cell. The skilled artisan appreciates the likelihood of successful gene expression if codon usage is biased towards those codons favored by the host. Determination of preferred codons can be based on a survey of genes derived from the host cell where sequence information is available.

“Gene” refers to a nucleic acid fragment that expresses a specific protein, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. “Native gene” refers to a gene as found in nature with its own regulatory sequences. “Chimeric gene” refers any gene that is not a native gene, comprising regulatory and coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. “Endogenous gene” refers to a native gene in its natural location in the genome of an organism. A “foreign” gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A “transgene” is a gene that has been introduced into the genome by a transformation procedure.

“Coding sequence” refers to a nucleotide sequence that codes for a specific amino acid sequence. “Regulatory sequences” refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

“Promoter” refers to a nucleotide sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. The promoter sequence consists of proximal and more distal upstream elements, the latter elements often referred to as enhancers. Accordingly, an “enhancer” is a nucleotide sequence which can stimulate promoter activity and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a promoter. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic nucleotide segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental conditions. Promoters which cause a nucleic acid fragment to be expressed in most cell types at most times are commonly referred to as “constitutive promoters”. New promoters of various types useful in plant cells are constantly being discovered; numerous examples may be found in the compilation by Okamuro and Goldberg (1989) *Biochemistry of Plants* 15:1-82. It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, nucleic acid fragments of different lengths may have identical promoter activity.

The “translation leader sequence” refers to a nucleotide sequence located between the promoter sequence of a gene and the coding sequence. The translation leader sequence is present in the fully processed mRNA upstream of the translation start sequence. The translation leader sequence may affect processing of the primary transcript to mRNA, mRNA stability or translation efficiency. Examples of translation leader sequences have been described (Turner and Foster (1995) *Mol. Biotechnol.* 3:225-236).

The “3' non-coding sequences” refer to nucleotide sequences located downstream of a coding sequence and include polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. The use of different 3' non-coding sequences is exemplified by Ingelbrecht et al. (1989) *Plant Cell* 1:671-680.

“RNA transcript” refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary

copy of the DNA sequence, it is referred to as the primary transcript or it may be a RNA sequence derived from posttranscriptional processing of the primary transcript and is referred to as the mature RNA. "Messenger RNA (mRNA)" refers to the RNA that is without introns and that can be translated into polypeptide by the cell. "cDNA" refers to a double-stranded DNA that is complementary to and derived from mRNA. "Sense" RNA refers to an RNA transcript that includes the mRNA and so can be translated into a polypeptide by the cell. "Antisense RNA" refers to an RNA transcript that is complementary to all or part of a target primary transcript or mRNA and that blocks the expression of a target gene (see U.S. Patent No. 5,107,065, incorporated herein by reference). The complementarity of an antisense RNA may be with any part of the specific nucleotide sequence, i.e., at the 5' non-coding sequence, 3' non-coding sequence, introns, or the coding sequence. "Functional RNA" refers to sense RNA, antisense RNA, ribozyme RNA, or other RNA that may not be translated but yet has an effect on cellular processes.

The term "operably linked" refers to the association of two or more nucleic acid fragments on a single nucleic acid fragment so that the function of one is affected by the other. For example, a promoter is operably linked with a coding sequence when it is capable of affecting the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in sense or antisense orientation.

The term "expression", as used herein, refers to the transcription and stable accumulation of sense (mRNA) or antisense RNA derived from the nucleic acid fragment of the invention. Expression may also refer to translation of mRNA into a polypeptide. "Antisense inhibition" refers to the production of antisense RNA transcripts capable of suppressing the expression of the target protein. "Overexpression" refers to the production of a gene product in transgenic organisms that exceeds levels of production in normal or non-transformed organisms. "Co-suppression" refers to the production of sense RNA transcripts capable of suppressing the expression of identical or substantially similar foreign or endogenous genes (U.S. Patent No. 5,231,020, incorporated herein by reference).

"Altered levels" refers to the production of gene product(s) in transgenic organisms in amounts or proportions that differ from that of normal or non-transformed organisms.

"Mature" protein refers to a post-translationally processed polypeptide; i.e., one from which any pre- or propeptides present in the primary translation product have been removed. "Precursor" protein refers to the primary product of translation of mRNA; i.e., with pre- and propeptides still present. Pre- and propeptides may be but are not limited to intracellular localization signals.

A "chloroplast transit peptide" is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the chloroplast or other plastid types present in the cell in which the protein is made. "Chloroplast transit sequence" refers to a

nucleotide sequence that encodes a chloroplast transit peptide. A "signal peptide" is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the secretory system (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol.* 42:21-53). If the protein is to be directed to a vacuole, a vacuolar targeting signal (*supra*) can further be added, or if to the endoplasmic reticulum, an endoplasmic reticulum retention signal (*supra*) may be added. If the protein is to be directed to the nucleus, any signal peptide present should be removed and instead a nuclear localization signal included (Raikhel (1992) *Plant Phys.* 100:1627-1632).

"Transformation" refers to the transfer of a nucleic acid fragment into the genome of a host organism, resulting in genetically stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as "transgenic" organisms. Examples of methods of plant transformation include *Agrobacterium*-mediated transformation (De Blaere et al. (1987) *Meth. Enzymol.* 143:277) and particle-accelerated or "gene gun" transformation technology (Klein et al. (1987) *Nature (London)* 327:70-73; U.S. Patent No. 4,945,050, incorporated herein by reference).

Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described more fully in Sambrook et al. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, 1989 (hereinafter "Maniatis").

Nucleic acid fragments encoding at least a portion of several vitamin E biosynthetic enzymes have been isolated and identified by comparison of random plant cDNA sequences to public databases containing nucleotide and protein sequences using the BLAST algorithms well known to those skilled in the art. The nucleic acid fragments of the instant invention may be used to isolate cDNAs and genes encoding homologous proteins from the same or other plant species. Isolation of homologous genes using sequence-dependent protocols is well known in the art. Examples of sequence-dependent protocols include, but are not limited to, methods of nucleic acid hybridization, and methods of DNA and RNA amplification as exemplified by various uses of nucleic acid amplification technologies (e.g., polymerase chain reaction, ligase chain reaction).

For example, genes encoding other 4-hydroxyphenylpyruvate dioxygenases or gamma tocopherol methyltransferases, either as cDNAs or genomic DNAs, could be isolated directly by using all or a portion of the instant nucleic acid fragments as DNA hybridization probes to screen libraries from any desired plant employing methodology well known to those skilled in the art. Specific oligonucleotide probes based upon the instant nucleic acid sequences can be designed and synthesized by methods known in the art (Maniatis). Moreover, the entire sequences can be used directly to synthesize DNA probes by methods known to the skilled artisan such as random primer DNA labeling, nick translation, or end-labeling techniques, or RNA probes using available *in vitro* transcription systems. In

addition, specific primers can be designed and used to amplify a part or all of the instant sequences. The resulting amplification products can be labeled directly during amplification reactions or labeled after amplification reactions, and used as probes to isolate full length cDNA or genomic fragments under conditions of appropriate stringency.

5 In addition, two short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols to amplify longer nucleic acid fragments encoding homologous genes from DNA or RNA. The polymerase chain reaction may also be performed on a library of cloned nucleic acid fragments wherein the sequence of one primer is derived from the instant nucleic acid fragments, and the sequence of the other primer takes  
10 advantage of the presence of the polyadenylic acid tracts to the 3' end of the mRNA precursor encoding plant genes. Alternatively, the second primer sequence may be based upon sequences derived from the cloning vector. For example, the skilled artisan can follow the RACE protocol (Frohman et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:8998-9002) to generate cDNAs by using PCR to amplify copies of the region between a single point in the  
15 transcript and the 3' or 5' end. Primers oriented in the 3' and 5' directions can be designed from the instant sequences. Using commercially available 3' RACE or 5' RACE systems (BRL), specific 3' or 5' cDNA fragments can be isolated (Ohara et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:5673-5677; Loh et al. (1989) *Science* 243:217-220). Products generated by the 3' and 5' RACE procedures can be combined to generate full-length cDNAs (Frohman  
20 and Martin (1989) *Techniques* 1:165). Consequently, a polynucleotide comprising a nucleotide sequence of at least one of 60 (preferably one of at least 40, most preferably one of at least 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and the complement of such nucleotide sequences may be used in such methods to  
25 obtain a nucleic acid fragment encoding a substantial portion of an amino acid sequence of a polypeptide. The present invention relates to a method of obtaining a nucleic acid fragment encoding a substantial portion of a polypeptide of a gene (such as gamma tocopherol methyltransferase or 4-phenylpyruvate dioxygenase) preferably a substantial portion of a plant polypeptide of a gene, comprising the steps of: synthesizing an oligonucleotide primer  
30 comprising a nucleotide sequence of at least one of 60 (preferably at least one of 40, most preferably at least one of 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, and the complement of such nucleotide sequences; and amplifying a nucleic acid fragment (preferably a cDNA inserted in a cloning vector) using the  
35 oligonucleotide primer. The amplified nucleic acid fragment preferably will encode a portion of a polypeptide (gamma tocopherol methyltransferase or 4-phenylpyruvate dioxygenase).

Availability of the instant nucleotide and deduced amino acid sequences facilitates immunological screening of cDNA expression libraries. Synthetic peptides representing portions of the instant amino acid sequences may be synthesized. These peptides can be used to immunize animals to produce polyclonal or monoclonal antibodies with specificity for peptides or proteins comprising the amino acid sequences. These antibodies can be then be used to screen cDNA expression libraries to isolate full-length cDNA clones of interest (Lerner (1984) *Adv. Immunol.* 36:1-34; Maniatis).

The nucleic acid fragments of the instant invention may be used to create transgenic plants in which the disclosed polypeptides are present at higher or lower levels than normal or in cell types or developmental stages in which they are not normally found. This would have the effect of altering the level of homogentate in those cells. Overexpression of 4-hydroxyphenylpyruvate dioxygenase should result in a larger accumulation of homogentate which may be used by gamma tocopherol methyltransferase to produce vitamin E. Since mammals can not synthesize tocopherols, the enzymes described herein may be used for the discovery of new herbicides.

Overexpression of the proteins of the instant invention may be accomplished by first constructing a chimeric gene in which the coding region is operably linked to a promoter capable of directing expression of a gene in the desired tissues at the desired stage of development. For reasons of convenience, the chimeric gene may comprise promoter sequences and translation leader sequences derived from the same genes. 3' Non-coding sequences encoding transcription termination signals may also be provided. The instant chimeric gene may also comprise one or more introns in order to facilitate gene expression.

Plasmid vectors comprising the instant chimeric gene can then be constructed. The choice of plasmid vector is dependent upon the method that will be used to transform host plants. The skilled artisan is well aware of the genetic elements that must be present on the plasmid vector in order to successfully transform, select and propagate host cells containing the chimeric gene. The skilled artisan will also recognize that different independent transformation events will result in different levels and patterns of expression (Jones et al. (1985) *EMBO J.* 4:2411-2418; De Almeida et al. (1989) *Mol. Gen. Genetics* 218:78-86), and thus that multiple events must be screened in order to obtain lines displaying the desired expression level and pattern. Such screening may be accomplished by Southern analysis of DNA, Northern analysis of mRNA expression, Western analysis of protein expression, or phenotypic analysis.

For some applications it may be useful to direct the instant polypeptides to different cellular compartments, or to facilitate its secretion from the cell. It is thus envisioned that the chimeric gene described above may be further supplemented by directing the coding sequence to encode the instant polypeptides with appropriate intracellular targeting sequences such as transit sequences (Keegstra (1989) *Cell* 56:247-253), signal sequences or

sequences encoding endoplasmic reticulum localization (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol.* 42:21-53), or nuclear localization signals (Raikhel (1992) *Plant Phys.* 100:1627-1632) with or without removing targeting sequences that are already present. While the references cited give examples of each of these, the list is not exhaustive and more  
5 targeting signals of use may be discovered in the future.

It may also be desirable to reduce or eliminate expression of genes encoding the instant polypeptides in plants for some applications. In order to accomplish this, a chimeric gene designed for co-suppression of the instant polypeptide can be constructed by linking a gene or gene fragment encoding that polypeptide to plant promoter sequences.

10 Alternatively, a chimeric gene designed to express antisense RNA for all or part of the instant nucleic acid fragment can be constructed by linking the gene or gene fragment in reverse orientation to plant promoter sequences. Either the co-suppression or antisense chimeric genes could be introduced into plants via transformation wherein expression of the corresponding endogenous genes are reduced or eliminated.

15 Molecular genetic solutions to the generation of plants with altered gene expression have a decided advantage over more traditional plant breeding approaches. Changes in plant phenotypes can be produced by specifically inhibiting expression of one or more genes by antisense inhibition or cosuppression (U.S. Patent Nos. 5,190,931, 5,107,065 and 5,283,323). An antisense or cosuppression construct would act as a dominant negative  
20 regulator of gene activity. While conventional mutations can yield negative regulation of gene activity these effects are most likely recessive. The dominant negative regulation available with a transgenic approach may be advantageous from a breeding perspective. In addition, the ability to restrict the expression of specific phenotype to the reproductive tissues of the plant by the use of tissue specific promoters may confer agronomic advantages  
25 relative to conventional mutations which may have an effect in all tissues in which a mutant gene is ordinarily expressed.

The person skilled in the art will know that special considerations are associated with the use of antisense or cosuppression technologies in order to reduce expression of particular genes. For example, the proper level of expression of sense or antisense genes may require  
30 the use of different chimeric genes utilizing different regulatory elements known to the skilled artisan. Once transgenic plants are obtained by one of the methods described above, it will be necessary to screen individual transgenics for those that most effectively display the desired phenotype. Accordingly, the skilled artisan will develop methods for screening large numbers of transformants. The nature of these screens will generally be chosen on  
35 practical grounds, and is not an inherent part of the invention. For example, one can screen by looking for changes in gene expression by using antibodies specific for the protein encoded by the gene being suppressed, or one could establish assays that specifically measure enzyme activity. A preferred method will be one which allows large numbers of

samples to be processed rapidly, since it will be expected that a large number of transformants will be negative for the desired phenotype.

The instant polypeptides (or portions thereof) may be produced in heterologous host cells, particularly in the cells of microbial hosts, and can be used to prepare antibodies to these proteins by methods well known to those skilled in the art. The antibodies are useful for detecting the polypeptides of the instant invention *in situ* in cells or *in vitro* in cell extracts. Preferred heterologous host cells for production of the instant polypeptides are microbial hosts. Microbial expression systems and expression vectors containing regulatory sequences that direct high level expression of foreign proteins are well known to those skilled in the art. Any of these could be used to construct a chimeric gene for production of the instant polypeptides. This chimeric gene could then be introduced into appropriate microorganisms via transformation to provide high level expression of the encoded vitamin E biosynthetic enzyme. An example of a vector for high level expression of the instant polypeptides in a bacterial host is provided (Example 7).

Additionally, the instant polypeptides can be used as a targets to facilitate design and/or identification of inhibitors of those enzymes that may be useful as herbicides. This is desirable because the polypeptides described herein catalyze various steps in vitamin E biosynthesis. Accordingly, inhibition of the activity of one or more of the enzymes described herein could lead to inhibition of plant growth. Thus, the instant polypeptides could be appropriate for new herbicide discovery and design.

All or a substantial portion of the nucleic acid fragments of the instant invention may also be used as probes for genetically and physically mapping the genes that they are a part of, and as markers for traits linked to those genes. Such information may be useful in plant breeding in order to develop lines with desired phenotypes. For example, the instant nucleic acid fragments may be used as restriction fragment length polymorphism (RFLP) markers. Southern blots (Maniatis) of restriction-digested plant genomic DNA may be probed with the nucleic acid fragments of the instant invention. The resulting banding patterns may then be subjected to genetic analyses using computer programs such as MapMaker (Lander et al. (1987) *Genomics* 1:174-181) in order to construct a genetic map. In addition, the nucleic acid fragments of the instant invention may be used to probe Southern blots containing restriction endonuclease-treated genomic DNAs of a set of individuals representing parent and progeny of a defined genetic cross. Segregation of the DNA polymorphisms is noted and used to calculate the position of the instant nucleic acid sequence in the genetic map previously obtained using this population (Botstein et al. (1980) *Am. J. Hum. Genet.* 32:314-331).

The production and use of plant gene-derived probes for use in genetic mapping is described in Bernatzky and Tanksley (1986) *Plant Mol. Biol. Reporter* 4:37-41. Numerous publications describe genetic mapping of specific cDNA clones using the methodology

outlined above or variations thereof. For example, F2 intercross populations, backcross populations, randomly mated populations, near isogenic lines, and other sets of individuals may be used for mapping. Such methodologies are well known to those skilled in the art.

5 Nucleic acid probes derived from the instant nucleic acid sequences may also be used for physical mapping (i.e., placement of sequences on physical maps; see Hoheisel et al. In: *Nonmammalian Genomic Analysis: A Practical Guide*, Academic press 1996, pp. 319-346, and references cited therein).

10 In another embodiment, nucleic acid probes derived from the instant nucleic acid sequences may be used in direct fluorescence *in situ* hybridization (FISH) mapping (Trask (1991) *Trends Genet.* 7:149-154). Although current methods of FISH mapping favor use of large clones (several to several hundred KB; see Laan et al. (1995) *Genome Res.* 5:13-20), improvements in sensitivity may allow performance of FISH mapping using shorter probes.

15 A variety of nucleic acid amplification-based methods of genetic and physical mapping may be carried out using the instant nucleic acid sequences. Examples include allele-specific amplification (Kazazian (1989) *J. Lab. Clin. Med.* 11:95-96), polymorphism of PCR-amplified fragments (CAPS; Sheffield et al. (1993) *Genomics* 16:325-332), allele-specific ligation (Landegren et al. (1988) *Science* 241:1077-1080), nucleotide extension reactions (Sokolov (1990) *Nucleic Acid Res.* 18:3671), Radiation Hybrid Mapping (Walter et al. (1997) *Nat. Genet.* 7:22-28) and Happy Mapping (Dear and Cook (1989) *Nucleic Acid*  
20 *Res.* 17:6795-6807). For these methods, the sequence of a nucleic acid fragment is used to design and produce primer pairs for use in the amplification reaction or in primer extension reactions. The design of such primers is well known to those skilled in the art. In methods employing PCR-based genetic mapping, it may be necessary to identify DNA sequence differences between the parents of the mapping cross in the region corresponding to the  
25 instant nucleic acid sequence. This, however, is generally not necessary for mapping methods.

Loss of function mutant phenotypes may be identified for the instant cDNA clones either by targeted gene disruption protocols or by identifying specific mutants for these genes contained in a maize population carrying mutations in all possible genes (Ballinger  
30 and Benzer (1989) *Proc. Natl. Acad. Sci USA* 86:9402-9406; Koes et al. (1995) *Proc. Natl. Acad. Sci USA* 92:8149-8153; Bensen et al. (1995) *Plant Cell* 7:75-84). The latter approach may be accomplished in two ways. First, short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols in conjunction with a mutation tag sequence primer on DNAs prepared from a population of plants in which  
35 Mutator transposons or some other mutation-causing DNA element has been introduced (see Bensen, *supra*). The amplification of a specific DNA fragment with these primers indicates the insertion of the mutation tag element in or near the plant gene encoding the instant polypeptides. Alternatively, the instant nucleic acid fragment may be used as a

hybridization probe against PCR amplification products generated from the mutation population using the mutation tag sequence primer in conjunction with an arbitrary genomic site primer, such as that for a restriction enzyme site-anchored synthetic adaptor. With either method, a plant containing a mutation in the endogenous gene encoding the instant polypeptides can be identified and obtained. This mutant plant can then be used to determine or confirm the natural function of the instant polypeptides disclosed herein.

### EXAMPLES

The present invention is further defined in the following Examples, in which all parts and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

#### EXAMPLE 1

##### Composition of cDNA Libraries; Isolation and Sequencing of cDNA Clones

cDNA libraries representing mRNAs from various corn, rice, soybean, *Vernonia*, and wheat tissues were prepared. The characteristics of the libraries are described below.

TABLE 2

cDNA Libraries from cDNA Libraries from Corn, Rice, Soybean, *Vernonia*, and Wheat

Library	Tissue	Clone
cr1n	Corn Root From 7 Day Old Seedlings*	cr1n.pk0155.d1
cr1n	Corn Root From 7 Day Old Seedlings*	cr1n.pk0179.f10
cs1	Corn Leaf Sheath From 5 Week Old Plant	cs1.pk0065.e1
cs1	Corn Leaf Sheath From 5 Week Old Plant	cs1.pk0065.f2
cta1n	Corn Tassel*	cta1n.pk0031.d2
ncs	<i>Catalpa speciosa</i> Developing Seed	ncs.pk0012.g1
p0023	Corn Leaf From Plant Transformed with Gene M1C07 (leucine-rich repeat) Which Induces Resistance Prior to Genetic Lesion Formation. Harvested About One Month After Planting in Green House*	p0060.coran49r
p0103	Corn Tassel Shoots(0.1-1.4 cm)*	p0103.ciaay86r
rl0n	Rice 15 Day Old Leaf*	rl0n.pk085.e11
rl0n	Rice 15 Day Old Leaf*	rl0n.pk099.d24
rlr12	Rice Leaf 15 Days After Germination, 12 Hours After Infection of Strain <i>Magaporthe grisea</i> 4360-R-62 (AVR2-YAMO); Resistant	rlr12.pk0019.g5

Library	Tissue	Clone
rlr12	Rice Leaf 15 Days After Germination, 12 Hours After Infection of Strain <i>Magaporthe grisea</i> 4360-R-62 (AVR2-YAMO); Resistant	rlr12.pk0025.e4
rls72	Rice Leaf 15 Days After Germination, 72 Hours After Infection of Strain <i>Magaporthe grisea</i> 4360-R-67 (AVR2-YAMO); Susceptible	rls72.pk0010.g3
sah1c	Soybean Sprayed With Authority Herbicide	sah1c.pk001.k8
sah1c	Soybean Sprayed With Authority Herbicide	sah1c.pk004.g2
sfl1	Soybean Immature Flower	sfl1.pk126.n15
sgc4c	Soybean Cotyledon 14-21 Days After Germination (1/4 yellow)	sgc4c.pk001.p2
sgc5c	Soybean (Cotyledon 15-24 Days After Germination (3/4 yellow)	sgc5c.pk001.j9
sgc5c	Soybean (Cotyledon 15-24 Days After Germination (3/4 yellow)	sgc5c.pk001.m23
sgs1c	Soybean Seeds 4 Hours After Germination	sgs1c.pk002.a8
sgs1c	Soybean Seeds 4 Hours After Germination	sgs1c.pk003.o5
vs1	<i>Vernonia</i> Seed Stage 1	vs1.pk0015.b2
wdk4c	Wheat Developing Kernel, 21 Days After Anthesis	wdk4c.pk006.m9
wle1n	Wheat Leaf From 7 Day Old Etiolated Seedling*	wle1n.pk0065.h9
wlm0	Wheat Seedlings 0 Hour After Inoculation With <i>Erysiphe graminis f. sp tritici</i>	wlm0.pk0035.d5
wlmk1	Wheat Seedlings 1 Hour After Inoculation With <i>Erysiphe graminis f. sp tritici</i> and Treatment With Herbicide**	wlmk1.pk0013.g11
wlmk1	Wheat Seedlings 1 Hour After Inoculation With <i>Erysiphe graminis f. sp tritici</i> and Treatment With Herbicide**	wlmk1.pk0019.e2
wlmk1	Wheat Seedlings 1 Hour After Inoculation With <i>Erysiphe graminis f. sp tritici</i> and Treatment With Herbicide**	wlmk1.pk0021.h10
wr1	Wheat Root From 7 Day Old Seedling	wr1.pk0077.f1
wr1	Wheat Root From 7 Day Old Seedling	wr1.pk177.b11
wre1n	Wheat Root From 7 Day Old Etiolated Seedling*	wre1n.pk0111.d6

\* These libraries were normalized essentially as described in U.S. Patent No. 5,482,845, incorporated herein by reference.

5 \*\* Application of 6-iodo-2-propoxy-3-propyl-4(3H)-quinazolinone; synthesis and methods of using this compound are described in USSN 08/545,827, incorporated herein by reference.

10 cDNA libraries may be prepared by any one of many methods available. For example, the cDNAs may be introduced into plasmid vectors by first preparing the cDNA libraries in Uni-ZAP™ XR vectors according to the manufacturer's protocol (Stratagene

Cloning Systems, La Jolla, CA). The Uni-ZAP™ XR libraries are converted into plasmid libraries according to the protocol provided by Stratagene. Upon conversion, cDNA inserts will be contained in the plasmid vector pBluescript. In addition, the cDNAs may be introduced directly into pre-cut Bluescript II SK(+) vectors (Stratagene) using T4 DNA  
5 ligase (New England Biolabs), followed by transfection into DH10B cells according to the manufacturer's protocol (GIBCO BRL Products). Once the cDNA inserts are in plasmid vectors, plasmid DNAs are prepared from randomly picked bacterial colonies containing recombinant pBluescript plasmids, or the insert cDNA sequences are amplified via  
10 polymerase chain reaction using primers specific for vector sequences flanking the inserted cDNA sequences. Amplified insert DNAs or plasmid DNAs are sequenced in dye-primer sequencing reactions to generate partial cDNA sequences (expressed sequence tags or "ESTs"; see Adams et al., (1991) *Science* 252:1651-1656). The resulting ESTs are analyzed using a Perkin Elmer Model 377 fluorescent sequencer.

### EXAMPLE 2

#### Identification of cDNA Clones

cDNA clones encoding vitamin E biosynthetic enzymes were identified by conducting BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol.* 215:403-410; see also [www.ncbi.nlm.nih.gov/BLAST/](http://www.ncbi.nlm.nih.gov/BLAST/)) searches for similarity to sequences contained in the BLAST "nr" database (comprising all non-redundant GenBank CDS  
20 translations, sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank, the last major release of the SWISS-PROT protein sequence database, EMBL, and DDBJ databases). The cDNA sequences obtained in Example 1 were analyzed for similarity to all publicly available DNA sequences contained in the "nr" database using the BLASTN  
25 algorithm provided by the National Center for Biotechnology Information (NCBI). The DNA sequences were translated in all reading frames and compared for similarity to all publicly available protein sequences contained in the "nr" database using the BLASTX algorithm (Gish and States (1993) *Nat. Genet.* 3:266-272) provided by the NCBI. For  
30 convenience, the P-value (probability) of observing a match of a cDNA sequence to a sequence contained in the searched databases merely by chance as calculated by BLAST are reported herein as "pLog" values, which represent the negative of the logarithm of the reported P-value. Accordingly, the greater the pLog value, the greater the likelihood that the cDNA sequence and the BLAST "hit" represent homologous proteins.

### EXAMPLE 3

#### Characterization of cDNA Clones Encoding

#### Gamma Tocopherol Methyltransferase

The BLASTX search using the EST sequences from clones listed in Table 3 revealed similarity of the polypeptides encoded by the cDNAs to gamma tocopherol methyltransferase from *Synechocystis* sp. (NCBI General Identifier No. 1001725). Shown in

Table 3 are the BLAST results for individual ESTs ("EST"), the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"), contigs assembled from two or more ESTs ("Contig"), contigs assembled from an FIS and one or more ESTs ("Contig\*"), or sequences encoding the entire protein derived from an FIS, a contig, or an FIS and PCR ("CGS"):

TABLE 3

BLAST Results for Sequences Encoding Polypeptides Homologous  
to gamma Tocopherol Methyltransferase

Clone	Status	BLAST pLog Score 1001725
Contig of: cr1n.pk0179.f10 cs1.pk0065.e1 cs1.pk0065.f2 cta1n.pk0031.d2	Contig	49.70
rl0n.pk085.e11	EST	15.10
Contig of: rl0n.pk099.d24 rls72.pk0010.g3	Contig	8.30
Contig of: sah1c.pk001.k8 sgc5c.pk001.m23 sah1c.pk004.g2:fis	CGS	64.22
Contig of: wr1.pk0077.f1 wr1.pk177.b11 wle1n.pk0065.h9 wre1n.pk0111.d6	Contig	57.00

10

Further sequencing and searching of the DuPont proprietary database allowed for the assembly of longer sequences. The BLASTX search using the nucleotide sequences from clones listed in Table 4 revealed similarity of the polypeptides encoded by the cDNAs to gamma tocopherol methyltransferase from *Arabidopsis thaliana* (NCBI General Identifier No. 4106538). Shown in Table 4 are the BLAST results for individual ESTs ("EST") or sequences encoding the entire protein derived from the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"), contigs assembled from an FIS and one or more ESTs, or an FIS and PCR ("CGS"):

15

TABLE 4

BLAST Results for Sequences Encoding Polypeptides Homologous  
to gamma Tocopherol Methyltransferase

Clone	Status	BLAST pLog Score 4106538
Contig of: crln.pk0179.f10:fis cs1.pk0065.f2 ctaln.pk0031.d2 p0060.coran49r p0103.ciaay86r	CGS	118.00
rls72.pk0010.g3-3'	EST*	81.30
rls72.pk0010.g3-5'	EST*	11.00
sahlc.pk004.g2	CGS	125.00
wrl.pk0077.f1:fis	CGS	120.00

5 \*These sequences are derived from partial full-insert sequencing of clone rls72.pk0010.g3  
and correspond to 5'-end and 3'-end sequences.

10 Figure 1 presents an alignment of the amino acid sequences set forth in SEQ ID  
NOs:8, 22, 24, 26, 28, and 30 and the *Synechocystis sp.* and *Arabidopsis thaliana* sequences  
(SEQ ID NO:39 and SEQ ID NO:40, respectively). The data in Table 5 represents a  
calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOs:8,  
22, 24, 26, 28, and 30 and the *Synechocystis sp.* and *Arabidopsis thaliana* sequences (SEQ  
ID NO:39 and SEQ ID NO:40, respectively).

TABLE 5

15 Percent Identity of Amino Acid Sequences Deduced From the Nucleotide  
Sequences of cDNA Clones Encoding Polypeptides Homologous  
to gamma Tocopherol Methyltransferase

SEQ ID NO.	Percent Identity to	
	1001725	4106538
8	37.9	56.9
22	42.6	58.6
24	42.8	67.6
26	23.8	33.3
28	41.3	62.6
30	43.2	59.5

20 Sequence alignments and percent identity calculations were performed using the  
Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc.,  
Madison, WI). Multiple alignment of the sequences was performed using the Clustal  
method of alignment (Higgins and Sharp (1989) *CABIOS*. 5:151-153) with the default

parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. Sequence alignments and BLAST scores and probabilities indicate that the nucleic acid fragments comprising the instant cDNA clones encode one entire corn, two entire soybean, one entire wheat, and two fragments corresponding to an almost entire rice gamma tocopherol methyltransferase. These sequences represent the first corn, rice, soybean, and wheat sequences encoding gamma tocopherol methyltransferase.

#### EXAMPLE 4

##### Characterization of cDNA Clones Encoding

##### 4-Hydroxyphenylpyruvate Dioxygenase

The BLASTX search using the EST sequences from clones listed in Table 6 revealed similarity of the polypeptides encoded by the cDNAs to 4-hydroxyphenylpyruvate dioxygenase from *Hordeum vulgare* (NCBI General Identifier No. 3334222), *Daucus carota* (NCBI General Identifier No. 3334219) and *Arabidopsis thaliana* (NCBI General Identifier No. 3334223). Shown in Table 6 are the BLAST results for individual ESTs ("EST") or contigs assembled from two or more ESTs ("Contig"):

TABLE 6

BLAST Results for Sequences Encoding Polypeptides Homologous to 4-Hydroxyphenylpyruvate Dioxygenase

Clone	Status	BLAST pLog Score		
		3334222	3334219	3334223
rlr12.pk0019.g5	EST	26.70	17.70	18.00
rlr12.pk0025.e4	EST	58.52	50.40	49.40
Contig of:	Contig	55.00	81.30	107.00
sgc5c.pk001.j9				
sgs1c.pk002.a8				
sfl1.pk126.n15				
sgs1c.pk003.o5				
sgc4c.pk001.p2				
vs1.pk0015.b2	EST	39.40	51.70	52.05
Contig of:	Contig	176.00	102.00	97.00
wdk4c.pk006.m9				
wlml1.pk0021.h1				
wlml1.pk0019.e2				
wlm0.pk0035.d5				
wlml1.pk0013.g1				

Further sequencing and searching of the DuPont proprietary database allowed the assembly of longer sequences and identification of 4-hydroxyphenylpyruvate dioxygenase in

other species. The BLASTX search using the nucleotide sequences from clones listed in Table 7 revealed similarity of the polypeptides encoded by the cDNAs to 4-hydroxyphenylpyruvate dioxygenase from *Hordeum vulgare* (NCBI General Identifier No. 3334222), *Daucus carota* (NCBI General Identifier No. 3334219) and *Arabidopsis thaliana* (NCBI General Identifier No. 3334223). Shown in Table 7 are the BLAST results for the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS") or sequences encoding the entire protein derived from an FIS, or an FIS and PCR ("CGS"):

TABLE 7

BLAST Results for Sequences Encoding Polypeptides Homologous to 4-Hydroxyphenylpyruvate Dioxygenase

Clone	Status	BLAST pLog Score		
		3334222	3334219	3334223
ncs.pk0012.g1:fis	CGS	147.00	254.00	177.00
rlr12.pk0025.e4:fis	FIS	117.00	102.00	100.00
sgc5c.pk001.j9:fis	CGS	149.00	>254.00	>254.00
wdk4c.pk006.m9:fis	CGS	>254.00	155.00	151.00

Figure 2 presents an alignment of the amino acid sequences set forth in SEQ ID NOs:32, 34, 36, and 38 and the *Hordeum vulgare*, *Daucus carota*, and *Arabidopsis thaliana* sequences (SEQ ID NO:41, SEQ ID NO:42, and SEQ ID NO:43, respectively). The data in Table 8 represents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOs:18, 32, 34, 36, and 38 and the *Hordeum vulgare*, *Daucus carota*, and *Arabidopsis thaliana* sequences (SEQ ID NO:41, SEQ ID NO:42, and SEQ ID NO:43, respectively)

TABLE 8

Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to 4-Hydroxyphenylpyruvate Dioxygenase

SEQ ID NO.	Percent Identity to		
	3334222	3334219	3334223
18	49.4	58.2	60.0
32	58.5	73.3	67.4
34	86.4	73.3	69.9
36	49.4	58.2	60.0
38	94.2	59.9	58.1

Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc.,

Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS*. 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. Sequence alignments and BLAST scores and probabilities indicate that the nucleic acid fragments comprising the instant cDNA clones encode substantial portions of rice, soybean, *Vernonia*, and wheat 4-hydroxyphenylpyruvate dioxygenase, and entire catalpa, soybean, and wheat 4-hydroxyphenylpyruvate dioxygenase. These sequences represent the first catalpa, rice, soybean, *Vernonia*, and wheat sequences encoding 4-hydroxyphenylpyruvate dioxygenase.

#### EXAMPLE 5

##### Expression of Chimeric Genes in Monocot Cells

A chimeric gene comprising a cDNA encoding the instant polypeptides in sense orientation with respect to the maize 27 kD zein promoter that is located 5' to the cDNA fragment, and the 10 kD zein 3' end that is located 3' to the cDNA fragment, can be constructed. The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites (NcoI or SmaI) can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the digested vector pML103 as described below. Amplification is then performed in a standard PCR. The amplified DNA is then digested with restriction enzymes NcoI and SmaI and fractionated on an agarose gel. The appropriate band can be isolated from the gel and combined with a 4.9 kb NcoI-SmaI fragment of the plasmid pML103. Plasmid pML103 has been deposited under the terms of the Budapest Treaty at ATCC (American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209), and bears accession number ATCC 97366. The DNA segment from pML103 contains a 1.05 kb Sali-NcoI promoter fragment of the maize 27 kD zein gene and a 0.96 kb SmaI-Sali fragment from the 3' end of the maize 10 kD zein gene in the vector pGem9Zf(+) (Promega). Vector and insert DNA can be ligated at 15°C overnight, essentially as described (Maniatis). The ligated DNA may then be used to transform *E. coli* XL1-Blue (Epicurian Coli XL-1 Blue™; Stratagene). Bacterial transformants can be screened by restriction enzyme digestion of plasmid DNA and limited nucleotide sequence analysis using the dideoxy chain termination method (Sequenase™ DNA Sequencing Kit; U.S. Biochemical). The resulting plasmid construct would comprise a chimeric gene encoding, in the 5' to 3' direction, the maize 27 kD zein promoter, a cDNA fragment encoding the instant polypeptides, and the 10 kD zein 3' region.

The chimeric gene described above can then be introduced into corn cells by the following procedure. Immature corn embryos can be dissected from developing caryopses derived from crosses of the inbred corn lines H99 and LH132. The embryos are isolated 10

to 11 days after pollination when they are 1.0 to 1.5 mm long. The embryos are then placed with the axis-side facing down and in contact with agarose-solidified N6 medium (Chu et al. (1975) *Sci. Sin. Peking* 18:659-668). The embryos are kept in the dark at 27°C. Friable embryogenic callus consisting of undifferentiated masses of cells with somatic proembryoids and embryoids borne on suspensor structures proliferates from the scutellum of these immature embryos. The embryogenic callus isolated from the primary explant can be cultured on N6 medium and sub-cultured on this medium every 2 to 3 weeks.

The plasmid, p35S/Ac (obtained from Dr. Peter Eckes, Hoechst Ag, Frankfurt, Germany) may be used in transformation experiments in order to provide for a selectable marker. This plasmid contains the *Pat* gene (see European Patent Publication 0 242 236) which encodes phosphinothricin acetyl transferase (PAT). The enzyme PAT confers resistance to herbicidal glutamine synthetase inhibitors such as phosphinothricin. The *pat* gene in p35S/Ac is under the control of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature* 313:810-812) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*.

The particle bombardment method (Klein et al. (1987) *Nature* 327:70-73) may be used to transfer genes to the callus culture cells. According to this method, gold particles (1  $\mu$ m in diameter) are coated with DNA using the following technique. Ten  $\mu$ g of plasmid DNAs are added to 50  $\mu$ L of a suspension of gold particles (60 mg per mL). Calcium chloride (50  $\mu$ L of a 2.5 M solution) and spermidine free base (20  $\mu$ L of a 1.0 M solution) are added to the particles. The suspension is vortexed during the addition of these solutions. After 10 minutes, the tubes are briefly centrifuged (5 sec at 15,000 rpm) and the supernatant removed. The particles are resuspended in 200  $\mu$ L of absolute ethanol, centrifuged again and the supernatant removed. The ethanol rinse is performed again and the particles resuspended in a final volume of 30  $\mu$ L of ethanol. An aliquot (5  $\mu$ L) of the DNA-coated gold particles can be placed in the center of a Kapton™ flying disc (Bio-Rad Labs). The particles are then accelerated into the corn tissue with a Biolistic™ PDS-1000/He (Bio-Rad Instruments, Hercules CA), using a helium pressure of 1000 psi, a gap distance of 0.5 cm and a flying distance of 1.0 cm.

For bombardment, the embryogenic tissue is placed on filter paper over agarose-solidified N6 medium. The tissue is arranged as a thin lawn and covered a circular area of about 5 cm in diameter. The petri dish containing the tissue can be placed in the chamber of the PDS-1000/He approximately 8 cm from the stopping screen. The air in the chamber is then evacuated to a vacuum of 28 inches of Hg. The macrocarrier is accelerated with a helium shock wave using a rupture membrane that bursts when the He pressure in the shock tube reaches 1000 psi.

Seven days after bombardment the tissue can be transferred to N6 medium that contains gluphosinate (2 mg per liter) and lacks casein or proline. The tissue continues to

grow slowly on this medium. After an additional 2 weeks the tissue can be transferred to fresh N6 medium containing glufosinate. After 6 weeks, areas of about 1 cm in diameter of actively growing callus can be identified on some of the plates containing the glufosinate-supplemented medium. These calli may continue to grow when sub-cultured on the selective medium.

Plants can be regenerated from the transgenic callus by first transferring clusters of tissue to N6 medium supplemented with 0.2 mg per liter of 2,4-D. After two weeks the tissue can be transferred to regeneration medium (Fromm et al. (1990) *Bio/Technology* 8:833-839).

#### EXAMPLE 6

##### Expression of Chimeric Genes in Dicot Cells

A seed-specific expression cassette composed of the promoter and transcription terminator from the gene encoding the  $\beta$  subunit of the seed storage protein phaseolin from the bean *Phaseolus vulgaris* (Doyle et al. (1986) *J. Biol. Chem.* 261:9228-9238) can be used for expression of the instant polypeptides in transformed soybean. The phaseolin cassette includes about 500 nucleotides upstream (5') from the translation initiation codon and about 1650 nucleotides downstream (3') from the translation stop codon of phaseolin. Between the 5' and 3' regions are the unique restriction endonuclease sites Nco I (which includes the ATG translation initiation codon), Sma I, Kpn I and Xba I. The entire cassette is flanked by Hind III sites.

The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the expression vector. Amplification is then performed as described above, and the isolated fragment is inserted into a pUC18 vector carrying the seed expression cassette.

Soybean embryos may then be transformed with the expression vector comprising sequences encoding the instant polypeptides. To induce somatic embryos, cotyledons, 3-5 mm in length dissected from surface sterilized, immature seeds of the soybean cultivar A2872, can be cultured in the light or dark at 26°C on an appropriate agar medium for 6-10 weeks. Somatic embryos which produce secondary embryos are then excised and placed into a suitable liquid medium. After repeated selection for clusters of somatic embryos which multiplied as early, globular staged embryos, the suspensions are maintained as described below.

Soybean embryogenic suspension cultures can be maintained in 35 mL liquid media on a rotary shaker, 150 rpm, at 26°C with florescent lights on a 16:8 hour day/night schedule. Cultures are subcultured every two weeks by inoculating approximately 35 mg of tissue into 35 mL of liquid medium.

Soybean embryogenic suspension cultures may then be transformed by the method of particle gun bombardment (Klein et al. (1987) *Nature* (London) 327:70-73, U.S. Patent No. 4,945,050). A DuPont Biolistic™ PDS1000/HE instrument (helium retrofit) can be used for these transformations.

- 5           A selectable marker gene which can be used to facilitate soybean transformation is a chimeric gene composed of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature* 313:810-812), the hygromycin phosphotransferase gene from plasmid pJR225 (from *E. coli*; Gritz et al.(1983) *Gene* 25:179-188) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*. The seed expression  
10 cassette comprising the phaseolin 5' region, the fragment encoding the instant polypeptides and the phaseolin 3' region can be isolated as a restriction fragment. This fragment can then be inserted into a unique restriction site of the vector carrying the marker gene.

- To 50 µL of a 60 mg/mL 1 µm gold particle suspension is added (in order): 5 µL DNA (1 µg/µL), 20 µl spermidine (0.1 M), and 50 µL CaCl<sub>2</sub> (2.5 M). The particle  
15 preparation is then agitated for three minutes, spun in a microfuge for 10 seconds and the supernatant removed. The DNA-coated particles are then washed once in 400 µL 70% ethanol and resuspended in 40 µL of anhydrous ethanol. The DNA/particle suspension can be sonicated three times for one second each. Five µL of the DNA-coated gold particles are then loaded on each macro carrier disk.

- 20           Approximately 300-400 mg of a two-week-old suspension culture is placed in an empty 60x15 mm petri dish and the residual liquid removed from the tissue with a pipette. For each transformation experiment, approximately 5-10 plates of tissue are normally bombarded. Membrane rupture pressure is set at 1100 psi and the chamber is evacuated to a vacuum of 28 inches mercury. The tissue is placed approximately 3.5 inches away from the  
25 retaining screen and bombarded three times. Following bombardment, the tissue can be divided in half and placed back into liquid and cultured as described above.

- Five to seven days post bombardment, the liquid media may be exchanged with fresh media, and eleven to twelve days post bombardment with fresh media containing 50 mg/mL hygromycin. This selective media can be refreshed weekly. Seven to eight weeks post  
30 bombardment, green, transformed tissue may be observed growing from untransformed, necrotic embryogenic clusters. Isolated green tissue is removed and inoculated into individual flasks to generate new, clonally propagated, transformed embryogenic suspension cultures. Each new line may be treated as an independent transformation event. These suspensions can then be subcultured and maintained as clusters of immature embryos or  
35 regenerated into whole plants by maturation and germination of individual somatic embryos.

### EXAMPLE 7

#### Expression of Chimeric Genes in Microbial Cells

The cDNAs encoding the instant polypeptides can be inserted into the T7 *E. coli* expression vector pBT430. This vector is a derivative of pET-3a (Rosenberg et al. (1987) *Gene* 56:125-135) which employs the bacteriophage T7 RNA polymerase/T7 promoter system. Plasmid pBT430 was constructed by first destroying the EcoR I and Hind III sites in pET-3a at their original positions. An oligonucleotide adaptor containing EcoR I and Hind III sites was inserted at the BamH I site of pET-3a. This created pET-3aM with additional unique cloning sites for insertion of genes into the expression vector. Then, the Nde I site at the position of translation initiation was converted to an Nco I site using oligonucleotide-directed mutagenesis. The DNA sequence of pET-3aM in this region, 5'-CATATGG, was converted to 5'-CCCATGG in pBT430.

Plasmid DNA containing a cDNA may be appropriately digested to release a nucleic acid fragment encoding the protein. This fragment may then be purified on a 1% NuSieve GTG™ low melting agarose gel (FMC). Buffer and agarose contain 10 µg/ml ethidium bromide for visualization of the DNA fragment. The fragment can then be purified from the agarose gel by digestion with GELase™ (Epicentre Technologies) according to the manufacturer's instructions, ethanol precipitated, dried and resuspended in 20 µL of water. Appropriate oligonucleotide adapters may be ligated to the fragment using T4 DNA ligase (New England Biolabs, Beverly, MA). The fragment containing the ligated adapters can be purified from the excess adapters using low melting agarose as described above. The vector pBT430 is digested, dephosphorylated with alkaline phosphatase (NEB) and deproteinized with phenol/chloroform as described above. The prepared vector pBT430 and fragment can then be ligated at 16°C for 15 hours followed by transformation into DH5 electrocompetent cells (GIBCO BRL). Transformants can be selected on agar plates containing LB media and 100 µg/mL ampicillin. Transformants containing the gene encoding the instant polypeptides are then screened for the correct orientation with respect to the T7 promoter by restriction enzyme analysis.

For high level expression, a plasmid clone with the cDNA insert in the correct orientation relative to the T7 promoter can be transformed into *E. coli* strain BL21(DE3) (Studier et al. (1986) *J. Mol. Biol.* 189:113-130). Cultures are grown in LB medium containing ampicillin (100 mg/L) at 25°C. At an optical density at 600 nm of approximately 1, IPTG (isopropylthio-β-galactoside, the inducer) can be added to a final concentration of 0.4 mM and incubation can be continued for 3 h at 25°. Cells are then harvested by centrifugation and re-suspended in 50 µL of 50 mM Tris-HCl at pH 8.0 containing 0.1 mM DTT and 0.2 mM phenyl methylsulfonyl fluoride. A small amount of 1 mm glass beads can be added and the mixture sonicated 3 times for about 5 seconds each time with a microprobe sonicator. The mixture is centrifuged and the protein concentration of the supernatant

determined. One  $\mu\text{g}$  of protein from the soluble fraction of the culture can be separated by SDS-polyacrylamide gel electrophoresis. Gels can be observed for protein bands migrating at the expected molecular weight.

### EXAMPLE 8

#### 5                    Evaluating Compounds for Their Ability to Inhibit the Activity    of Vitamin E Biosynthetic Enzymes

10                    The polypeptides described herein may be produced using any number of methods known to those skilled in the art. Such methods include, but are not limited to, expression in bacteria as described in Example 7, or expression in eukaryotic cell culture, *in planta*, and using viral expression systems in suitably infected organisms or cell lines. The instant polypeptides may be expressed either as mature forms of the proteins as observed *in vivo* or as fusion proteins by covalent attachment to a variety of enzymes, proteins or affinity tags. Common fusion protein partners include glutathione S-transferase ("GST"), thioredoxin ("Trx"), maltose binding protein, and C- and/or N-terminal hexahistidine polypeptide ("His<sub>6</sub>"). The fusion proteins may be engineered with a protease recognition site at the fusion point so that fusion partners can be separated by protease digestion to yield intact mature enzyme. Examples of such proteases include thrombin, enterokinase and factor Xa. However, any protease can be used which specifically cleaves the peptide connecting the fusion protein and the enzyme.

20                    Purification of the instant polypeptides, if desired, may utilize any number of separation technologies familiar to those skilled in the art of protein purification. Examples of such methods include, but are not limited to, homogenization, filtration, centrifugation, heat denaturation, ammonium sulfate precipitation, desalting, pH precipitation, ion exchange chromatography, hydrophobic interaction chromatography and affinity chromatography, wherein the affinity ligand represents a substrate, substrate analog or inhibitor. When the instant polypeptides are expressed as fusion proteins, the purification protocol may include the use of an affinity resin which is specific for the fusion protein tag attached to the expressed enzyme or an affinity resin containing ligands which are specific for the enzyme. For example, the instant polypeptides may be expressed as a fusion protein coupled to the C-terminus of thioredoxin. In addition, a (His)<sub>6</sub> peptide may be engineered into the N-terminus of the fused thioredoxin moiety to afford additional opportunities for affinity purification. Other suitable affinity resins could be synthesized by linking the appropriate ligands to any suitable resin such as Sepharose-4B. In an alternate embodiment, a thioredoxin fusion protein may be eluted using dithiothreitol; however, elution may be accomplished using other reagents which interact to displace the thioredoxin from the resin. These reagents include  $\beta$ -mercaptoethanol or other reduced thiol. The eluted fusion protein may be subjected to further purification by traditional means as stated above, if desired. Proteolytic cleavage of the thioredoxin fusion protein and the enzyme may be accomplished

after the fusion protein is purified or while the protein is still bound to the ThioBond™ affinity resin or other resin.

Crude, partially purified or purified enzyme, either alone or as a fusion protein, may be utilized in assays for the evaluation of compounds for their ability to inhibit enzymatic activation of the instant polypeptides disclosed herein. Assays may be conducted under well known experimental conditions which permit optimal enzymatic activity. For example, assays for gamma tocopherol methyltransferase are presented by d'Harlingue and Camara (1985) *J. Biol. Chem.* 260:15200-15203. Assays for 4-hydroxyphenylpyruvate dioxygenase are presented by Norris et al. (1995) *Plant Cell* 7: 2139-2149.

#### EXAMPLE 9

##### Expression of gamma Tocopherol Methyltransferase in Soybean Somatic Embryos

The ability to change the levels of alpha- and gamma-tocopherol in plants by transforming them with sequences encoding gamma tocopherol methyltransferase was tested by preparing transgenic soybean somatic embryos and assaying the tocopherol levels. Plasmid DNA from clone sahl c.pk001.k8 was used as a template to prepare a Not I pcr fragment encoding the entire deduced open reading frame using the following pcr primers (forward primer AGC GCG GCC GCA TGG CCA CCG TGG TGA GGA TCC CAA CAA TCT CAT GCA TCC ACA TCC ACA; reverse primer AGC GCG GCC GCT TAT CTA GTG TGG AAA TAA TGA TCA). Standard pcr reactions were used (100 microliter total reaction containing 5 ng plasmid, 25 nmoles primer, 25 nmoles dNTPs, 1x cloned pfu buffer (Stratagene), 5% DMSO, 5U cloned pfu DNA polymerase (Stratagene). The recommended cycling parameters for pcr with pfu DNA polymerase were used (denature 45 s 94°C, anneal 45 s 55°C, extend 2.5 min 72°C, 25 cycles, final 10min extension at 72°C). The pcr product was purified on a 1% agarose/TAE gel (precast FMC), the ethidium bromide visualized band cut out and purified using a QIAquick gel extraction kit (Qiagen). The band prep (40 ng) was ligated into PCR Blunt (InVitrogen) according to the manufacturers recommendations, and the ligated plasmid used to transform *E. coli* DH10 cells. Kanamycin resistant colonies were grown overnight in liquid culture (LB/Kan), plasmids prepared and cut with Not I. Plasmids containing the correct insert size were selected for full insert sequence to confirm fidelity of pcr. Plasmids containing the correct insert verified by DNA sequencing were digested with Not I and ligated to Not I-digested and phosphatase-treated pKS67. The plasmid pKS67 was prepared by replacing in pRB20 (described in U.S. Patent No. 5,846,784) the 800 bp Nos 3' fragment, with the 285 bp Nos 3' fragment containing the polyadenylation signal sequence and described in Depicker et al. (1982) *J. Mol. Appl. Genet.* 1:561-573. Clones were screened for the sense and anti-sense orientation of the gamma tocopherol methyltransferase insert fragment by restriction enzyme digestion.

*Transformation of Soybean Somatic Embryo Cultures*

The following stock solutions and media were used for transformation and propagation of soybean somatic embryos:

Stock Solutions		Media
<u>MS Sulfate 100x stock</u>		<u>SB55 (per Liter)</u>
	(g/L)	
MgSO <sub>4</sub> .7H <sub>2</sub> O	37.0	10 mL of each MS stock
MnSO <sub>4</sub> .H <sub>2</sub> O	1.69	1 mL of B5 Vitamin stock
ZnSO <sub>4</sub> .7H <sub>2</sub> O	0.86	0.8 g NH <sub>4</sub> NO <sub>3</sub>
CuSO <sub>4</sub> .5H <sub>2</sub> O	0.0025	3.033 g KNO <sub>3</sub>
		1 mL 2,4-D (10 mg/mL stock)
		0.667 g asparagine
		pH 5.7
<u>MS Halides 100x stock</u>		<u>SB103 (per Liter)</u>
CaCl <sub>2</sub> .2H <sub>2</sub> O	44.0	1 pk. Murashige & Skoog salt mixture*
KI	0.083	60 g maltose
CoCl <sub>2</sub> .6H <sub>2</sub> O	0.00125	2 g gelrite
KH <sub>2</sub> PO <sub>4</sub>	17.0	pH 5.7
H <sub>3</sub> BO <sub>3</sub>	0.62	
Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O	0.025	
Na <sub>2</sub> EDTA	3.724	
FeSO <sub>4</sub> .7H <sub>2</sub> O	2.784	
		<u>SB148 (per Liter)</u>
<u>B5 Vitamin stock</u>		1 pk. Murashige & Skoog salt mixture*
myo-inositol	100.0	60 g maltose
nicotinic acid	1.0	1 mL B5 vitamin stock
pyridoxine HCl	1.0	7 g agarose
thiamine	10.0	pH 5.7

5

\*(Gibco BRL)

Soybean embryonic suspension cultures were maintained in 35 mL liquid media (SB55) on a rotary shaker (150 rpm) at 28°C with a mix of fluorescent and incandescent lights providing a 16 h day 8 h night cycle. Cultures were subcultured every 2 to 3 weeks by inoculating approximately 35 mg of tissue into 35 mL of fresh liquid media.

Soybean embryonic suspension cultures were transformed with the plasmid containing the gamma tocopherol methyltransferase sequence by the method of particle gun bombardment (see Klein et al. (1987) *Nature* 327:70-73) using a DuPont Biolistic PDS1000/He instrument. Five µL of pKS93s plasmid DNA (1 g/L), 50 µL CaCl<sub>2</sub> (2.5 M), and 20 µL spermidine (0.1 M) were added to 50 µL of a 60 mg/mL 1 mm gold particle suspension. The particle preparation was agitated for 3 minutes, spun on a microfuge for

10 seconds and the supernate removed. The DNA-coated particles were then washed once with 400  $\mu$ L of 70% ethanol and resuspended in 40  $\mu$ L of anhydrous ethanol. The DNA/particle suspension was sonicated three times for 1 second each. Five  $\mu$ L of the DNA-coated gold particles were then loaded on each macro carrier disk.

- 5        Approximately 300 to 400 mg of two-week-old suspension culture was placed in an empty 60 mm X 15 mm petri dish and the residual liquid removed from the tissue using a pipette. The tissue was placed about 3.5 inches away from the retaining screen and bombarded twice. Membrane rupture pressure was set at 1100 psi and the chamber was evacuated to -28 inches of Hg. Two plates were bombarded, and following bombardment,  
10       the tissue was divided in half, placed back into liquid media, and cultured as described above.

- Fifteen days after bombardment, the liquid media was exchanged with fresh SB55 containing 50 mg/mL hygromycin. The selective media was refreshed weekly. Six weeks after bombardment, green, transformed tissue was isolated and inoculated into flasks to  
15       generate new transformed embryonic suspension cultures.

- Transformed embryonic clusters were removed from liquid culture media and placed on a solid agar media, SB103, containing 0.5% charcoal to begin maturation. After 1 week, embryos were transferred to SB103 media minus charcoal. After 5 weeks on SB103 media, maturing embryos were separated and placed onto SB148 media. During maturation  
20       embryos were kept at 26°C with a mix of fluorescent and incandescent lights providing a 16 h day 8 h night cycle. After 3 weeks on SB148 media, embryos were analyzed for the expression of the tocopherols. Each embryonic cluster gave rise to 5 to 20 somatic embryos.

      Non-transformed somatic embryos were cultured by the same method as used for the transformed somatic embryos.

#### 25       *Analysis of Transformed Somatic Embryos*

- At the end of the 6<sup>th</sup> week on SB148 medium somatic embryos were harvested from 25 independently transformed lines. Somatic embryos were collected in pools of five and weighed for fresh weight. Excess embryos were stored in 96-well plates at -80°. The pooled somatic embryos were lyophilized for 18 hours and the dry weight measured. The  
30       lyophilized somatic embryos were briefly pulverised with a hand held Potter homogeniser and then 600  $\mu$ L of heptane added and the samples incubated for 24 hours in the dark at room temperature to extract oils and tocopherols. The heptane was decanted and a further 300  $\mu$ L added to the samples. The extracts were combined and centrifuged (5 min, 12000 g). The supernatant was stored in amber hplc autosampler vials at -20°C prior to analysis.

- 35       HPLC analysis of the extracts was carried out using an HP1100 system (Agilent Technologies) 25  $\mu$ L of the heptane sample was applied to a Lichrosphere Si 60 column (5 micron 4 x 12.5 mm). The column was eluted with heptane/isopropanol (98:2 v/v) at a flow rate of 1 mL/min. After 6 minutes all four tocopherol isomers were eluted, as detected by

a HP1100 fluorescence detector (Excitation wavelength 295 nm, emission wavelength 330 nm). Individual tocopherol standards (Matreya) were diluted with hplc grade heptane to levels between 1 and 200 ng/ul to construct a 6 point external standard curve. Tocopherols in each oil were quantified using a standard curve run on the same day as the samples. The

5 sum of tocopherol peak areas of samples from a non-transformed control line were compared with those of 25 independent gamma tocopherol methyltransferase-transformed, hygromycin resistant lines. Figure 3 shows a graph depicting the distribution of the percent alpha- and gamma-tocopherol of soybean somatic embryos transgenic for the gamma tocopherol methyltransferase gene and a control line. The mean alpha tocopherol content is 40 to 70%.

10 Some lines, such as the ones represented in bars 2, 5, and 18 appear to represent overexpression of the gamma tocopherol methyltransferase, which would be predicted to yield higher levels of alpha tocopherol. Other lines, such as those represented in bars 6 and 10 appear to represent co-suppression of the gamma tocopherol methyltransferase since this is predicted to yield higher levels of gamma tocopherol. These results indicate that

15 transgenic expression of gamma-tocopherol methyltransferase affords the ability to manipulate tocopherol levels as desired for a particular application.

Various modifications of the invention in addition to those shown and described herein will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

20 The disclosure of each reference set forth above is incorporated herein by reference in its entirety.

## CLAIMS

What is claimed is:

1. An isolated polynucleotide comprising a first nucleotide sequence encoding a polypeptide of at least 160 amino acids that has at least 80% identity based on the Clustal  
5 method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 22, 24, 26, 28, and 30, or a second nucleotide sequence comprising the complement of the first nucleotide sequence.
2. An isolated polynucleotide comprising a first nucleotide sequence encoding a polypeptide of at least 150 amino acids that has at least 95% identity based on the Clustal  
10 method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:12, 14, 16, 18, 20, 32, 34, 36, and 38, or a second nucleotide sequence comprising the complement of the first nucleotide sequence.
3. The isolated polynucleotide of Claim 1 or Claim 2, wherein the first nucleotide sequence consists of a nucleic acid sequence selected from the group consisting of SEQ ID  
15 NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, and 37 that codes for the polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, and 38.
4. The isolated polynucleotide of Claim 1 or Claim 2 wherein the nucleotide sequences are DNA.
- 20 5. The isolated polynucleotide of Claim 1 or Claim 2 wherein the nucleotide sequences are RNA.
6. A chimeric gene comprising the isolated polynucleotide of Claim 1 or Claim 2 operably linked to suitable regulatory sequences.
7. An isolated host cell comprising the chimeric gene of Claim 6.
- 25 8. A host cell comprising an isolated polynucleotide of Claim 1 or Claim 2.
9. The host cell of Claim 8 wherein the host cell is selected from the group consisting of yeast, bacteria, plant, and virus.
10. A virus comprising the isolated polynucleotide of Claim 1 or Claim 2.
11. A polypeptide of at least 160 amino acids that has at least 80% identity based on  
30 the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 22, 24, 26, 28, and 30.
12. A polypeptide of at least 150 amino acids that has at least 95% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:12, 14, 16, 18, 20, 32, 34, 36, and 38.
- 35 13. A method of selecting an isolated polynucleotide that affects the level of expression of a vitamin E biosynthetic enzyme polypeptide in a plant cell, the method comprising the steps of:

(a) constructing an isolated polynucleotide comprising a nucleotide sequence of at least one of 30 contiguous nucleotides derived from an isolated polynucleotide of Claim 1 or Claim 2;

(b) introducing the isolated polynucleotide into a plant cell; and

5 (c) measuring the level of a polypeptide in the plant cell containing the polynucleotide to provide a positive selection means.

14. The method of Claim 13 wherein the isolated polynucleotide consists of a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, and 37 that codes for the polypeptide selected from  
10 the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, and 38.

15. A method of selecting an isolated polynucleotide that affects the level of expression of a vitamin E biosynthetic enzyme polypeptide in a plant cell, the method comprising the steps of:

15 (a) constructing an isolated polynucleotide of Claim 1 or Claim 2;

(b) introducing the isolated polynucleotide into a plant cell; and

(c) measuring the level of polypeptide in the plant cell containing the polynucleotide to provide a positive selection means.

16. A method of obtaining a nucleic acid fragment encoding a vitamin E  
20 biosynthetic enzyme polypeptide comprising the steps of:

(a) synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least one of 30 contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, and the complement of such nucleotide sequences; and

25 (b) amplifying a nucleic acid sequence using the oligonucleotide primer.

17. A method of obtaining a nucleic acid fragment encoding a vitamin E biosynthetic enzyme polypeptide comprising the steps of:

(a) probing a cDNA or genomic library with an isolated polynucleotide comprising at least one of 30 contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and the complement of such nucleotide sequences;

(b) identifying a DNA clone that hybridizes with the isolated polynucleotide;

(c) isolating the identified DNA clone; and

35 (d) sequencing the cDNA or genomic fragment that comprises the isolated DNA clone.

18. A method for evaluating at least one compound for its ability to inhibit the activity of a vitamin E biosynthetic enzyme, the method comprising the steps of:

(a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding a vitamin E biosynthetic enzyme, operably linked to suitable regulatory sequences;

5 (b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of the vitamin E biosynthetic enzyme encoded by the operably linked nucleic acid fragment in the transformed host cell;

(c) optionally purifying the vitamin E biosynthetic enzyme expressed by the transformed host cell;

10 (d) treating the vitamin E biosynthetic enzyme with a compound to be tested; and

(e) determining the activity of the vitamin E biosynthetic enzyme that has been treated with the compound, thereby selecting compounds with potential for inhibitory activity.

15 19. A composition comprising the isolated polynucleotide of Claim 1 or Claim 2.

20. A composition comprising the isolated polypeptide of Claim 11.

21. An isolated polynucleotide comprising the nucleotide sequence having at least one of 30 contiguous nucleotides derived from a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 20 35, 37 and the complement of such sequences.

22. An expression cassette comprising an isolated polynucleotide of Claim 1 or Claim 2 operably linked to a promoter.

23. A method for positive selection of a transformed cell comprising:

25 (a) transforming a host cell with an expression cassette of Claim 22; and

(b) growing the transformed host cell under conditions which allow expression of the polynucleotide in an amount sufficient to complement a null mutant to provide a positive selection means.

24. The method of Claim 23 wherein the plant cell is a monocot.

25. The method of Claim 23 wherein the plant cell is a dicot.

30

## FIGURE 1

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SEQ ID NO:39  MVYHVRPKHAL-----FLAFYCYFS-----
SEQ ID NO:40  MKATLAAPSSLTSL-P-----YRTNSS-FGSKSSLLFRSPSSSSSVS-MTTTRGNV
SEQ ID NO:38  MAN-----SXRPA-PLTPLHLRLDAAPPRLPSLGHAAARPVPRPVLPLPAR-XLRAPDGV
SEQ ID NO:28  MATVVRI-----PTISCIHIHTRSQSPRTFARIRVGPRSWAPIRASA-ASSERGEI
SEQ ID NO:08  MATVVRI-----PTISCIHIHTRSQSPRTFARIRVGPRSWAPIRASA-ASSERGEI
SEQ ID NO:22  MAHAALLHCSQSSR-SLAACRRGSHYRAPSH-VPRHSRRLRAVVSL---R-PMASSTA-
SEQ ID NO:24  AR--VQPTGALAPLHPLLRCTSRHLCASASPRAGLCLHHRRRRRRSSRRTKLAVRAMAPT
SEQ ID NO:26  FRH-----GH-----AL-----
1
60

SEQ ID NO:39  LLTMASATIASADLYEKIKNFYDDSSGLWEDVWGEHMHGYYGPHGTYRI---DRRQAQI
SEQ ID NO:40  AVAAAATSTEAA--LRKGIAEFYNETSGLWEIWDHMHGIFYDPDSSVQLSDSGHKEAQI
SEQ ID NO:38  VDDRGPGDAAPPGLKEGIAGLYDESSGLWESIWEHMHGIFYDSGEAASMSD--HRRAQI
SEQ ID NO:28  VLEQPKKKDDKKLQKIAEFYDESSGLWENIWDHMHGIFYDSDTVSLSD--HRAAQI
SEQ ID NO:08  VLEQPKKKDDKKLQKIAEFYDESSGLWENIWDHMHGIFYDSDTVSLSD--HRAAQI
SEQ ID NO:22  ----QAPATAPPGLKEGIAGLYDESSGLWENIWDHMHGIFYDSSEAASMSD--HRRAQI
SEQ ID NO:24  LSSSSTAAAPPGLKEGIAGLYDESSGVWESIWEHMHGIFYDAGEAASMSD--HRRAQI
61
120

SEQ ID NO:39  DLIKELLAWAVPQNSA----KPRKILDLCGGIGGSSLLYLAQQHQAQAEVVGASLSPVQVERA
SEQ ID NO:40  RMIEESLRFAGVTDEEE-EKKIKKVVDVCGGIGGSSRYLASKFGAECIGITLSPVQAKRA
SEQ ID NO:38  RMIEEALAFAAVP--DDPTNKPTIVDVCGGIGGSSRYLANKYGAQCSCGITLSPVQAERG
SEQ ID NO:28  RMIQESLRFASV--SEERSKWPKSIVDVCGGIGGSSRYLAKKFGATSVGITLSPVQAQRA
SEQ ID NO:08  RMIQESLRFASV--SEERSKWPKSIVDVCGGIGGSSRYLAKKFGATSVGITLSPVQAQRA
SEQ ID NO:22  RMIEEALAFAGVPASDDPEKTPKTIVDVCGGIGGSSRYLAKKYGXQCTGITLSPVQAERG
SEQ ID NO:24  RMIEESLAF-----
121
180

SEQ ID NO:26  -----

```

## FIGURE 1

```

SEQ ID NO:39 GERARALGLSTCQFQVANALDLPFASDSFDWVWSLESGEHMPNKAQFLQEAWRVLKPGG
SEQ ID NO:40 NDIAAAQSLSHKASFQVADALDQPFEDGKFDLVWSMESGEHMPDKAKFVKELVRVAAPGG
SEQ ID NO:38 NALAAAQGLSDKASFQVADALEQFPDGGQFDLVWSMESGEHMPNKKFVSELARVAAPGA
SEQ ID NO:28 NALAAAQGLADKVSFQVADALQQPFSDGQFDLVWSMESGEHMPDKAKFVGELARVAAPGA
SEQ ID NO:08 NALAAAQGLADKVSFQVADALQQPFSDGQFDLVWSMESGEHMPDKAKFVGELARVAAPGA
SEQ ID NO:22 NALAAAQGLSDQVTLQVADALEQFPDGGQFDLVWSMESGEHMPDKRKFFVSELARVAAPGG
SEQ ID NO:24 -----
SEQ ID NO:26 -----AQFPDGGQFDLVWSMESDEHMPDKRQFVSELARVAAPGA
181 240

SEQ ID NO:39 RLILATWCHRPIDPGNGPLTADERRHLQAIYDVYCLPYVVSPLPDYEAIARECGFGEIKTA
SEQ ID NO:40 RIIIVTWCHRNLSAGEEALQPWEQNILDKICKTFYLPAWCSTDDYVNLQSHSLQDIKCA
SEQ ID NO:38 TIIIVTWCHRNLPASEDSLKPDELNLLKKICDAYYLPDWCSPSDYVKIAESLSLEDIKTA
SEQ ID NO:28 IIIIVTWCHRD LGPDEQSLHPWEQD LKKICDAYYLPAWCSTSDYVKLLQSLSLQDIKSE
SEQ ID NO:08 IIIIVTWCHRD LGPDEQSLHPWEQD LKKICDAYYLPAWCSTSDYVKLLQSLSLQDIKSE
SEQ ID NO:22 TIIIVTWCHRNLDPSETSLKPDELSLRRICDAYYLPDWCSPSDYVNIAKSLSLEDIKTA
SEQ ID NO:24 -----
SEQ ID NO:26 RIIIVTWCHRNLEPSEESLKPDELNLLKRICDAYYLPDWCSPSDYVKIAESLSLEDIRTA
241 300

SEQ ID NO:39 DWSVAVAPFWDRVIESAFDPRVLWALGQAGPKIINAALCLRLMKWYERGLVRFGLLTGI
SEQ ID NO:40 DWSENVAPFWPAVIRTALTWKGLVSLRSGMKSIKGALTMPLMIEGYKKGVIKFGIITCQ
SEQ ID NO:38 DWSENVAPFWPAVIOALTWKGLTSLRSGWKTIKGALVMPMLIQGYKKGLIKFSIITCR
SEQ ID NO:28 DWSRFVAPFWPAVIRSAFTWKGLSLLSSGQTIKGALAMPLMIEGYKKDLIKFAIITCR
SEQ ID NO:08 DWSRFVAPFWPAVIRSAFTWKGLSLLSSGKGLI-----YIAFQKQTPPSSIATCK
SEQ ID NO:22 DWSENVAPFWPAVIRSAFTWKGLTSLTTGWKTIRGAMVMPLMIQGYKKGLIKFTIITCR
SEQ ID NO:24 -----
SEQ ID NO:26 DWSENVAPFWPAVIRSAFTWKGLTSLRSGWETVRGAMVMPLVIEGYKKGLIKFPFIITCR
301 360

```

## FIGURE 1

```
SEQ ID NO:39 KPLV-----  
SEQ ID NO:40 KPL-----  
SEQ ID NO:38 KPQAAIEGEPEAAPSVE.-  
SEQ ID NO:28 K-----PE-----  
SEQ ID NO:08 SYVTDHYFTR.-----  
SEQ ID NO:22 KPGAA-.MA-----HAALLHC  
SEQ ID NO:24 -----  
SEQ ID NO:26 KPETT-----Q.-  
361 380
```

## FIGURE 2

SEQ ID NO: 41 MPPTPTTAAATGAAAAVTPEHARPH-----RMVRENPRSDREHTLSEHHVEFWCADAASA  
 SEQ ID NO: 43 MGHQNAAVSENONHDDGAASSPGFKLVGFSKFVRKNPKSDKFKVKRFHHIEFWCGDATNV  
 SEQ ID NO: 42 MGKKQSEAEILSS-NSSNTSPATFKLVGFNNFVRANPKSDHFAVKRFHHIEFWCGDATNT  
 SEQ ID NO: 36 MPIPMCNEIQAQA-QAQAQAQPGFKLVGEKNEFVRTNPKSDRFQVNRFFHHIEFWCTDATNA  
 SEQ ID NO: 38 MPPTPTTAAATGAAA-VTPEHARPR-----RMVRENPRSDREHTLAFHHVEFWCADAASA  
 SEQ ID NO: 32 MGK-QTTTSATAA-DGSKDAHAEFKLVGKNEFVRTNPKSDHFCVHRFFHHIEFWCGDATNT  
 1 60

SEQ ID NO: 41 AGRFALGAPLAARSDLSTGNSAHASQLLRSGSLAFLFTAPYANG-----CDAATASL  
 SEQ ID NO: 43 ARRFSGWGLGMRFSAKSDLSTGNMVMHASLYLLTSGDLRFLFTAPYSPSLSAGEIKPTTTASI  
 SEQ ID NO: 42 SRRFSWGLGMPPLVAKSDLSTGNSVHASLYLRSANLSEFVFTAPYSPSTTSS-G---SAAI  
 SEQ ID NO: 36 SRRFSWGLGMPPIVAKSDLSTGNQIHASLYLLRSGDLSELFSAFYSPSLSAGS-SAASSASI  
 SEQ ID NO: 38 AGRFALGAPLAARSDLSTGNSVHASQLLRSGNLAFLFTAPYANG-----CDAATASL  
 SEQ ID NO: 32 AKRFSWGLGMPPLVAKSDLSTGNSAHASLYLLRSGELNFLTSPYSPSISAPS-----SAAI  
 61 120

SEQ ID NO: 41 PSFSADAARRFSADHGIAVRSVALRVADAAEAFRASRRRGARPAFAPVDLGRGFA-FAEV  
 SEQ ID NO: 43 PSFDHGSCRSFFSSHGLGVRAVAIEVEDAESAFSISVANGAIPSSPPIVLNEAVT-IAEV  
 SEQ ID NO: 42 PSFSASGFHSFAAKHGLAVRAIALEVADVAAAFASVARGARPASAPVEL-DDQAWLAEV  
 SEQ ID NO: 36 PSFDAATCLAFAAKHGFGVRAIALEVADAAEAFAASVAKGAEPASPPV-LVDDRTGFAEV  
 SEQ ID NO: 38 PSFSADAARQFSADHGLAVRSIALRVADAAEAFRASVDGGARPAFSPVDLGRGFG-FAEV  
 SEQ ID NO: 32 PSFSFSTYQSFSTSSHGLAVRAVAIQVDSAFSAYSASISRGAKPVSAPIILLSDNKTIAEV  
 121 180

## FIGURE 2

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SEQ ID NO:41  ELYGDEVVLRVFSH-PDGTDP-----FLPGFEGVTNPD-----VDYGLTRFDHVVGNVP      240
SEQ ID NO:43  KLYGDEVVLRVVSYKAEDTEKSE-----FLPGFERVEDASSFP-LDYGIRRLDHAVGNVP
SEQ ID NO:42  ELYGDEVVLRVFSFGRE-----EGLFLPGFEAVEGTASFPDLDYGIRRLDHAVGNVT
SEQ ID NO:36  RLYGDEVVLRVVSYKDAAPQAPHADPSRWELPGFEAAASSSSFPELDYGIRRLDHAVGNVP
SEQ ID NO:38  ELYGDEVVLRVFSH-PDGRDVP-----FLPGFEGVSNPD-----VDYGLTRFDHVVGNVP
SEQ ID NO:32  HLYGDSVLRVFSYGDNG-----TGPDGWFLPGFEPVDDQMSYKELDYGIRRLDHAVGNVP      181
                                                    241

SEQ ID NO:41  ELAPAAAYIAGFTGFHEFAEFTAEDVGTESGLNSVVLANNSEGVLLPLNEPVHGTKRRS
SEQ ID NO:43  ELGPALTYVAGFTGFHQFAEFTADDVGTAESGLNSAVLASNDEMVLPLPINEPVHGTKRKS
SEQ ID NO:42  ELGPVVEYIKGFTGFHEFAEFTAEDVGTLESGLSVVLANNSEMVLLPLNEPVYGTKRKS
SEQ ID NO:36  ELAPAVRYLKGFGFHEFAEFTAEDVGTSEGLNSVVLANNSETVLLPLNEPVYGTKRKS
SEQ ID NO:38  ELAPAAAYVAGFTGFHEFAEFTTEDVGTAESGLNSMVLANNSEGVLLPLNEPVHGTKRRS
SEQ ID NO:32  ELGPVVDYLLKKFTGFHEFAEFTSEDVGTAESGLNSMVLANNENVLLPLNEPVFGTKRKS      241
                                                    300

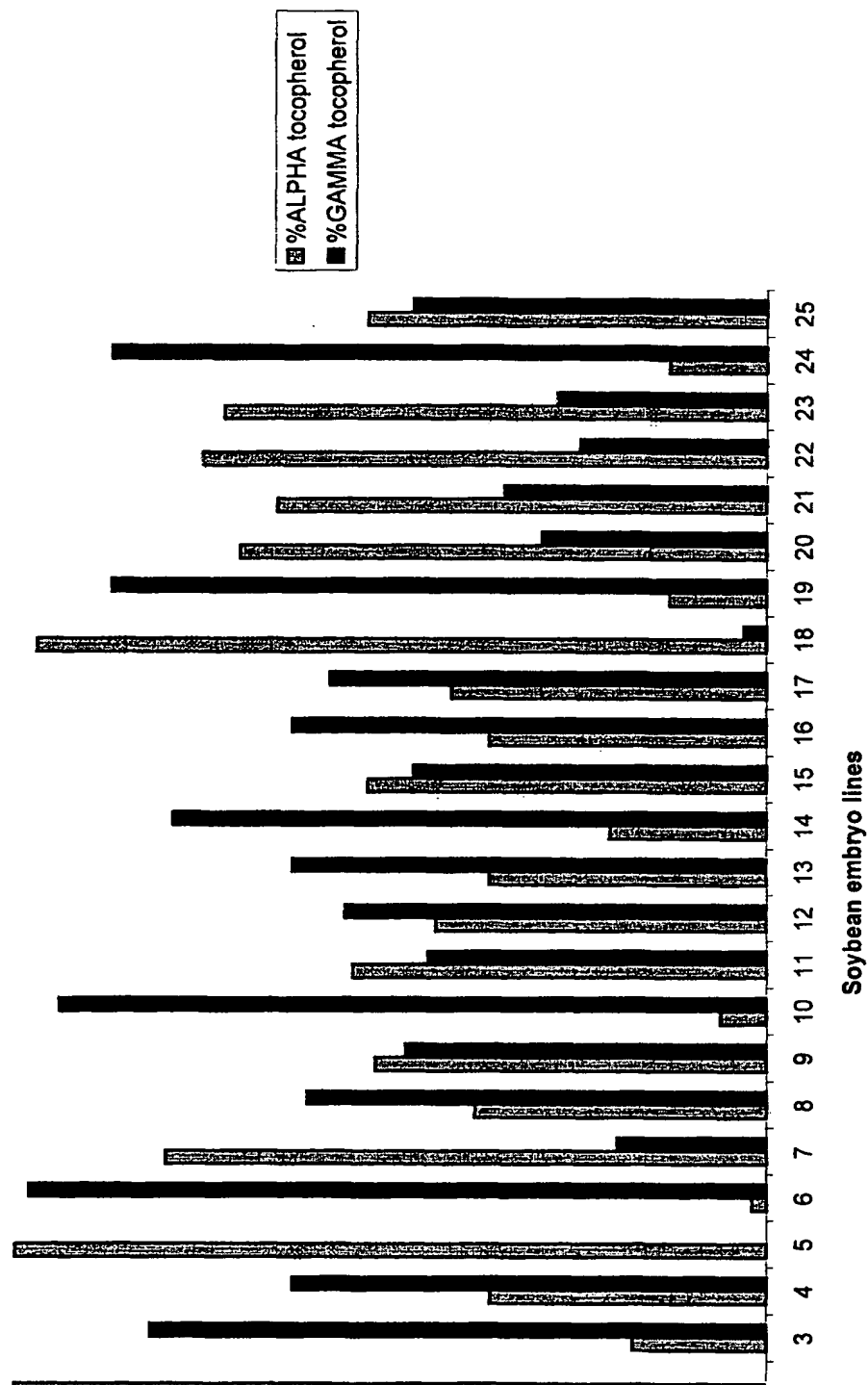
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SEQ ID NO:38  QIQTFLEHHGGGVQVQHIAVASSDVLRTLREMRARSAMGGFDLPPPLPKYYEGVRRRIAGD
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## FIGURE 2

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SEQ ID NO: 32	VLRDEQIEECEEKLGILVDRDDQGTLLQIFTKPVGDRPTLFIEIIQIRIGCMKDEQGKLYQ	420
		361
SEQ ID NO: 41	KGCGGFGKGNFSELFKSIEDYEKSLEAKQSAAVQG-S	
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SEQ ID NO: 42	KGCGGFGKGNFSELFKSIEEYEKTLQAKQITGSA-AA	
SEQ ID NO: 36	KGACGGFGKGNFSELFKSIEEYEKTLQAKR-----TA.	
SEQ ID NO: 38	KGCGGFGKGNFSELFKSIEDYEKSLEAKQSAAVQGS.	
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Figure 3



SEQUENCE LISTING

<110> Rafalski, J. Antoni  
 Cahoon, Rebecca E.  
 Coughlan, Sean  
 Miao, Guo-Hua

<120> PLANT VITAMIN E BIOSYNTHETIC ENZYMES

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<151> 1998-12-03

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<213> Zea mays

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<212> PRT

<213> Zea mays

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Gly Leu Ser Asp Gln Val Thr Leu Gln Val Ala Asp Ala Leu Glu Gln
      20             25             30

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Pro Phe Pro Asp Gly Gln Phe Asp Leu Val Trp Ser Met Glu Ser Gly
      35             40             45

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Glu His Met Pro Asp Lys Arg Lys Phe Val Ser Glu Leu Ala Arg Val
      50             55             60

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Leu Asp Pro Ser Glu Thr Ser Leu Lys Pro Asp Glu Leu Ser Leu Leu  
 85 90 95

Arg Arg Ile Cys Asp Ala Tyr Tyr Leu Pro Asp Trp Cys Ser Pro Ser  
 100 105 110

Asp Tyr Val Asn Ile Ala Lys Ser Leu Ser Leu Glu Asp Ile Lys Thr  
 115 120 125

Ala Asp Trp Ser Glu Asn Val Ala Pro Phe Trp Pro Ala Val Ile Lys  
 130 135 140

Ser Ala Leu Thr Trp Lys Gly Phe Thr Ser Leu Leu Thr Thr Gly Trp  
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Lys Lys Gly Leu Ile Lys Phe Thr Ile Ile Thr Cys Arg Lys Pro  
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 20 25 30

Pro Ser Glu Glu Ser Leu Lys Pro Asp Glu Leu Asn Leu Leu Lys Arg  
 35 40 45

Ile Cys Asp Ala Tyr Tyr Leu Pro Asp Trp Cys Ser Pro Ser Asp Tyr  
 50 55 60

Val Lys Ile Ala Glu Ser Leu Ser Leu Glu Asp Ile Arg Thr Ala Asp  
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 35 40 45  
 Leu Cys Leu His His His Arg Arg Arg Arg Ser Ser Arg Arg Thr  
 50 55 60  
 Lys Leu Ala Val Arg Ala Met Ala Pro Thr Leu Ser Ser Ser Ser Thr  
 65 70 75 80  
 Ala Ala Ala Ala Pro Pro Gly Leu Lys Glu Gly Ile Ala Gly Leu Tyr  
 85 90 95  
 Asp Glu Xaa Ser Gly Val Trp Glu Ser Ile Trp Gly Glu His Met His  
 100 105 110  
 His Gly Phe Tyr Asp Ala Gly Glu Gly Ala Ser Met Ser Asp His Arg  
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&lt;211&gt; 349

&lt;212&gt; PRT

&lt;213&gt; Glycine max

&lt;400&gt; 8

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20          25          30

Pro Arg Ser Trp Ala Pro Ile Arg Ala Ser Ala Ala Ser Ser Glu Arg
35          40          45

Gly Glu Ile Val Leu Glu Gln Lys Pro Lys Lys Asp Asp Lys Lys Lys
50          55          60

Leu Gln Lys Gly Ile Ala Glu Phe Tyr Asp Glu Ser Ser Gly Leu Trp
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Glu Asn Ile Trp Gly Asp His Met His His Gly Phe Tyr Asp Ser Asp
85          90          95

Ser Thr Val Ser Leu Ser Asp His Arg Ala Ala Gln Ile Arg Met Ile
100         105         110

Gln Glu Ser Leu Arg Phe Ala Ser Val Ser Glu Glu Arg Ser Lys Trp
115         120         125

Pro Lys Ser Ile Val Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg
130         135         140

Tyr Leu Ala Lys Lys Phe Gly Ala Thr Ser Val Gly Ile Thr Leu Ser
145         150         155         160

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Pro Val Gln Ala Gln Arg Ala Asn Ala Leu Ala Ala Ala Gln Gly Leu  
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Ser Asp Gly Gln Phe Asp Leu Val Trp Ser Met Glu Ser Gly Glu His  
 195 200 205

Met Pro Asp Lys Ala Lys Phe Val Gly Glu Leu Ala Arg Val Ala Ala  
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Pro Gly Ala Ile Ile Ile Ile Val Thr Trp Cys His Arg Asp Leu Gly  
 225 230 235 240

Pro Asp Glu Gln Ser Leu His Pro Trp Glu Gln Asp Leu Leu Lys Lys  
 245 250 255

Ile Cys Asp Ala Tyr Tyr Leu Pro Ala Trp Cys Ser Thr Ser Asp Tyr  
 260 265 270

Val Lys Leu Leu Gln Ser Leu Ser Leu Gln Asp Ile Lys Ser Glu Asp  
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Trp Ser Arg Phe Val Ala Pro Phe Trp Pro Ala Val Ile Arg Ser Ala  
 290 295 300

Phe Thr Trp Lys Gly Leu Ser Ser Leu Leu Ser Ser Gly Lys Leu Gly  
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Ile Tyr Ile Ala Phe Gln Lys Gln Thr Pro Pro Ser Ser Ile Ala Thr  
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 Ala Gln Ile Arg Met Ile Glu Glu Ala Leu Ala Phe Ala Ala Val Pro  
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 Asp Asp Pro Thr Asn Lys Pro Lys Thr Ile Val Asp Val Gly Cys Gly  
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 Ile Gly Gly Ser Ser Arg Tyr Leu Gly Glu Gln Ile Trp Ser Thr Met  
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 Leu Trp Asp His Ile Asp Pro Val Gln Ala Glu Arg Gly Asn Ala Leu  
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 Ala Ala Ala Gln Gly Val Val Arg Thr Arg Phe Phe Pro Ile Ala Asp  
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 Leu Trp Glu Gln Pro Phe Pro Gly Trp Ala Phe Asp Leu Val Xaa Xaa  
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 Xaa Xaa Xaa Xaa Xaa His Met Pro Asn Lys Gln Lys Phe Val Ser Glu  
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     145                    150                    155                    160  
 Cys His Arg Asn Leu Ala Pro Ser Glu Asp Ser Leu Lys Pro Asp Glu  
             165                    170                    175  
 Leu Asn Leu Leu Lys Lys Ile Cys Asp Ala Tyr Tyr Leu Pro Asp Trp  
             180                    185                    190  
 Cys Ser Pro Ser Asp Tyr Val Lys Ile Ala Glu Ser Leu Ser Leu Glu  
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 Asp Ile Lys Thr Ala Asp Trp Ser Glu Asn Val Ala Pro Phe Trp Pro  
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 Ala Val Ile Gln Ser Ala Leu Thr Trp Lys Gly Leu Thr Ser Leu Leu  
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 Arg Ser Gly Trp Lys Thr Ile Lys Gly Ala Leu Val Met Pro Leu Met  
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 Ile Gln Gly Tyr Lys Lys Gly Leu Ile Lys Phe Lys His His His Leu  
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35 40 45  
Phe Leu Phe Thr Ala Pro Tyr Gly Gly Asp His Gly Val Gly Ala Asp  
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&lt;213&gt; Oryza sativa

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 20 25 30

Ala Glu Phe Thr Ala Glu Asp Val Gly Thr Ala Glu Ser Gly Leu Asn  
 35 40 45

Ser Val Val Leu Ala Asn Asn Ala Glu Thr Val Leu Leu Pro Leu Asn  
 50 55 60

Glu Pro Val His Gly Thr Lys Arg Arg Ser Gln Ile Gln Thr Tyr Leu  
 65 70 75 80

Asp His His Gly Gly Pro Gly Val Gln His Ile Ala Leu Ala Ser Asp  
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Asp Val Leu Gly Thr Leu Xaa Glu Met Pro Gly Ala Ser Ala Trp Ala  
 100 105 110

Val Arg Phe Leu Gly Pro Pro Pro Pro Thr Thr  
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&lt;211&gt; 1027

&lt;212&gt; DNA

&lt;213&gt; Glycine max

&lt;400&gt; 15

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&lt;211&gt; 276

&lt;212&gt; PRT

&lt;213&gt; Glycine max

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 Arg Thr Asn Pro Lys Ser Asp Arg Phe Gln Val Asn Arg Phe His His  
 35 40 45  
 Ile Glu Phe Trp Cys Thr Asp Ala Thr Asn Ala Ser Arg Arg Phe Ser  
 50 55 60  
 Trp Gly Leu Gly Met Pro Ile Val Ala Lys Ser Asp Leu Ser Thr Gly  
 65 70 75 80  
 Asn Gln Ile His Ala Ser Tyr Leu Leu Arg Ser Gly Asp Leu Ser Phe  
 85 90 95  
 Leu Phe Ser Ala Pro Tyr Ser Pro Ser Leu Ser Ala Gly Ser Ser Ala  
 100 105 110  
 Ala Ser Ser Ala Ser Ile Pro Ser Phe Asp Ala Ala Thr Cys Leu Ala  
 115 120 125  
 Phe Ala Ala Lys His Gly Phe Gly Val Arg Ala Ile Ala Leu Glu Val  
 130 135 140  
 Ala Asp Ala Glu Ala Ala Phe Ser Ala Ser Val Ala Lys Gly Ala Glu  
 145 150 155 160  
 Pro Ala Ser Pro Pro Val Leu Val Asp Asp Arg Thr Gly Phe Ala Glu  
 165 170 175  
 Val Arg Leu Tyr Gly Asp Val Val Leu Arg Tyr Val Ser Tyr Lys Asp  
 180 185 190  
 Ala Ala Pro Gln Ala Pro His Ala Asp Xaa Ser Arg Trp Phe Leu Pro  
 195 200 205  
 Gly Phe Glu Ala Ala Ala Ser Ser Ser Ser Phe Pro Glu Leu Asp Tyr  
 210 215 220  
 Gly Ile Arg Arg Leu Asp His Ala Val Gly Asn Val Pro Glu Leu Ala  
 225 230 235 240  
 Pro Ala Val Arg Tyr Leu Lys Gly Phe Ser Gly Phe His Glu Phe Ala  
 245 250 255  
 Glu Phe Thr Ala Glu Asp Val Gly Thr Ser Glu Ser Gly Leu Asn Ser  
 260 265 270  
 Val Val Leu Ala  
 275

<210> 17  
 <211> 511  
 <212> DNA  
 <213> *Vernonia mesipifolia*

<400> 17  
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 cgctgctcct gtaacccttg gaaacaacga cgctgtattg tctgaagtta agctttacgg 180  
 cgatgtcgct ttccggtaca taagttacaa aaatccgaac tatacatctt cttttttgcc 240  
 cgggttcgag cccgttgaaa agacgtcgct gttttatgac cttgactacg gtatccgccc 300  
 tttggaccac gccgtaggaa cgccctgag cttgcttcgg cagtggacta cgtgaaatca 360  
 ttcaccggat tccatgagtt cgccgaattc accgcggagg acgtcggggac gagcgagagg 420  
 gaactgaatt cggtcgtttt agcttgcaac agtgagatgg tcttgattcc gatgaacgag 480  
 ccggtgtacg gaanaaaagg aagagccaga t 511

<210> 18  
 <211> 170  
 <212> PRT  
 <213> Vernonia mesipifolia

<400> 18  
 His Thr Asp Cys Arg Asn Phe Thr Ala Ser His Gly Leu Ala Val Arg  
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 20 25 30  
 Val Ser His Gly Ala Lys Pro Ser Ala Ala Pro Val Thr Leu Gly Asn  
 35 40 45  
 Asn Asp Val Val Leu Ser Glu Val Lys Leu Tyr Gly Asp Val Ala Phe  
 50 55 60  
 Arg Tyr Ile Ser Tyr Lys Asn Pro Asn Tyr Thr Ser Ser Phe Leu Pro  
 65 70 75 80  
 Gly Phe Glu Pro Val Glu Lys Thr Ser Ser Phe Tyr Asp Leu Asp Tyr  
 85 90 95  
 Gly Ile Arg Arg Leu Asp His Ala Val Gly Asn Val Pro Glu Leu Ala  
 100 105 110  
 Ser Ala Val Asp Tyr Val Lys Ser Phe Thr Gly Phe His Glu Phe Ala  
 115 120 125  
 Glu Phe Thr Ala Glu Asp Val Gly Thr Ser Glu Arg Glu Leu Asn Ser  
 130 135 140  
 Val Val Leu Ala Cys Asn Ser Glu Met Val Leu Ile Pro Met Asn Glu  
 145 150 155 160  
 Pro Val Tyr Gly Xaa Lys Gly Arg Ala Arg  
 165 170

<210> 19  
 <211> 1165  
 <212> DNA  
 <213> Triticum aestivum

<400> 19  
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 ccgcttccac acgtcgcct tccaccacgt cgagttctgg tgcgcggacg ccgcctccgc 180  
 cgccggccgc ttcgccttcg cgctcggcgc gccgtcgcc gccaggtccg acctctccac 240

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ggggaactcc gtgcacgcct cccagctgct ccgctcgggc aacctcgctt tcctcttcac 300
cgcgccctac gccaacggct gcgacgcgcg caccgcctcc ctgccctcct tctccgcoga 360
cgccgcgcgc cggttctccg cggaccacgg gctcgcggtg cgtcccatag cgctgcgcgt 420
cgcgagacgc gccgaggcct tccgcgccag cgtcgacggg ggcgcgcgcc cggccttcag 480
ccccgtggac ctccggccgcg gcttcgggtt tgcggaggtc gagctctacg gcgacgtcgt 540
gctccgcttc gtcagcatcc ggacggcnac gacgtgcctt cttgccgggg ttcgangggc 600
ttgagcaacc ggggtgccgtg gactaanggc tgacacggnt tgacacgttg tccgnaagtc 660
cggagcttgc ttcggcgccg cctaacgtag ccggctnaac gggttcaana attcgccagt 720
taacacggag gacgtgggca cggccgagag cgggctcaac tcgatggtgc tcgccaacaa 780
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gatacagacg ttcctggaac accacggcgg ctcgggctg cagcacatcg cgggtggccag 900
cagcgacgtg ctcaggacgc tcaggagagc gcgtgcgcgc tccgccatgg cgggcttcga 960
cttcctgcca ccccgcgtgc cgaagtacta cgaaggcgtg cggcgcatcg cgggggatgt 1020
gctctcggag gcgcaaatna aggaatgcaa gaactggggg tgctcntcca caaggaagaa 1080
caaaggtgtg tgctacaaat cctcaacaag ccaatntggg acaagccgac ttgttctctg 1140
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&lt;210&gt; 20

&lt;211&gt; 179

&lt;212&gt; PRT

&lt;213&gt; Triticum aestivum

&lt;400&gt; 20

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Met Pro Pro Thr Pro Thr Thr Pro Ala Ala Thr Gly Ala Ala Ala Val
 1              5              10              15

Thr Pro Glu His Ala Arg Pro Arg Arg Met Val Arg Phe Asn Pro Arg
      20              25              30

Ser Asp Arg Phe His Thr Leu Ala Phe His His Val Glu Phe Trp Cys
      35              40              45

Ala Asp Ala Ala Ser Ala Ala Gly Arg Phe Ala Phe Ala Leu Gly Ala
      50              55              60

Pro Leu Ala Ala Arg Ser Asp Leu Ser Thr Gly Asn Ser Val His Ala
      65              70              75              80

Ser Gln Leu Leu Arg Ser Gly Asn Leu Ala Phe Leu Phe Thr Ala Pro
      85              90              95

Tyr Ala Asn Gly Cys Asp Ala Ala Thr Ala Ser Leu Pro Ser Phe Ser
      100              105              110

Ala Asp Ala Ala Arg Arg Phe Ser Ala Asp His Gly Leu Ala Val Arg
      115              120              125

Ser Ile Ala Leu Arg Val Ala Asp Ala Ala Glu Ala Phe Arg Ala Ser
      130              135              140

Val Asp Gly Gly Ala Arg Pro Ala Phe Ser Pro Val Asp Leu Gly Arg
      145              150              155              160

Gly Phe Gly Phe Ala Glu Val Glu Leu Tyr Gly Asp Val Val Leu Arg
      165              170              175

Phe Val Ser

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<210> 21  
 <211> 1102  
 <212> DNA  
 <213> Zea mays

<220>  
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 <222> (454)

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<220>  
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 gccgtcgta gccgtcgctcc gatggcctcg tcgacggctc aggccccgc gacggcgccg 180  
 ccgggtctga aggagggcat cgcggggctg tacgacgagt cgtcggggct gtgggagaac 240  
 atctggggcg accacatgca ccacggcttc tacgactcga gcgaggccgc ctccatggcc 300  
 gatcaccgcc gcgccagat ccgcatgatc gaggaggcgc tcgccttcgc cgggtgtccca 360  
 gcctcagatg atccagagaa gacacaaaa acaatagtcg atgtcggatg tggcattggt 420  
 ggtagctcaa ggtacttggc gaagaaatac ggancgcagt gcaactggat cacgttgagc 480  
 cctgttcaag ccgagagagg aaatgctctc gctgcagcgc aggggttgtc ggatcagggt 540  
 actctgcaag ttgctgatgc tctggagcaa ccgtttcctg acgggcagtt cgatctggtg 600  
 tgggtccatgg agagtggcga gcacatgccg gacaagagaa agtttgtag tgagctagca 660  
 cgcgtggcgg ctccctggagg gacaataatc atcgtgacat ggtgccatag gaacctggat 720  
 ccatccgaaa cctcgctaaa gcccgatgaa ctgagcctcc tgaggaggat atgcgacgcg 780  
 tactacctcc cggactggtg ctcaccttca gactatgtga acattgcca gtcactgtct 840  
 ctgaggata tcaagacagc tgactggctc gagaacgtgg ccccgttttg gcccgcctg 900  
 ataaaatcag cgtaacatg gaagggttc acctctctgc tgacgaccgg atggaagacg 960  
 atcagaggcg cgatggtgat gccgctaata atccagggt acaagaagg gctcatcaaa 1020  
 ttcacatca tcacctgtcg caagcctgga gccgcgtagt gatctatacc gnccacggcg 1080  
 tcnttaactc tnacggaan ct 1102

<210> 22  
 <211> 352  
 <212> PRT  
 <213> Zea mays

<220>  
 <221> UNSURE  
 <222> (152)

<400> 22  
 Met Ala His Ala Ala Leu Leu His Cys Ser Gln Ser Ser Arg Ser Leu  
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13

Gly Leu Ile Lys Phe Thr Ile Ile Thr Cys Arg Lys Pro Gly Ala Ala  
                   340                                  345                                  350

<210> 23  
 <211> 521  
 <212> DNA  
 <213> Oryza sativa

<220>  
 <221> unsure  
 <222> (269)

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<220>  
 <221> unsure  
 <222> (514)

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 ataatcattg tgacctggtg ccataggaac ctcgagccat ccgaagagtc cctgaaacct 120  
 gatgagctga atctcctgaa aaggatatgc gatgcatatt atctcccaga ctgggtgctct 180  
 ccttctgatt atgtcaaaat tgccgagtc ctgtctcttg aggatataag gacagctgat 240  
 tgggtcaagag aacgtcgccc caatccggnc tgcnggttat taaatnaagc aattgacatg 300  
 gnaagggtta actttctcct ggctaagaan tgggtgggaa gacgattaag aagggtggaat 360  
 ggggtgatgcc tccggatgat nnaaggntac aaagaaangg gtcaacaaat ttaacaanaa 420  
 caacctgtnc caaagncccg aaacaacgca ataatacccc antaatnaaa ttncgctcct 480  
 ggctaacctt ctccaacaac gaattaatgg aaantttctga c 521

<210> 24  
 <211> 172  
 <212> PRT  
 <213> Oryza sativa

<400> 24  
 Phe Arg His Gly His Ala Leu Ala Gln Pro Phe Pro Asp Gly Gln Phe  
 1 5 10 15  
 Asp Leu Val Trp Ser Met Glu Ser Asp Glu His Met Pro Asp Lys Arg  
 20 25 30  
 Gln Phe Val Ser Glu Leu Ala Arg Val Ala Ala Pro Gly Ala Arg Ile  
 35 40 45  
 Ile Ile Val Thr Trp Cys His Arg Asn Leu Glu Pro Ser Glu Glu Ser  
 50 55 60  
 Leu Lys Pro Asp Glu Leu Asn Leu Leu Lys Arg Ile Cys Asp Ala Tyr  
 65 70 75 80  
 Tyr Leu Pro Asp Trp Cys Ser Pro Ser Asp Tyr Val Lys Ile Ala Glu  
 85 90 95  
 Ser Leu Ser Leu Glu Asp Ile Arg Thr Ala Asp Trp Ser Glu Asn Val  
 100 105 110  
 Ala Pro Phe Trp Pro Ala Val Ile Lys Ser Ala Leu Thr Trp Lys Gly  
 115 120 125  
 Leu Thr Ser Leu Leu Arg Ser Gly Trp Glu Thr Val Arg Gly Ala Met  
 130 135 140  
 Val Met Pro Leu Val Ile Glu Gly Tyr Lys Lys Gly Leu Ile Lys Phe  
 145 150 155 160  
 Pro Ile Ile Thr Cys Arg Lys Pro Glu Thr Thr Gln  
 165 170

<210> 25  
 <211> 464

<212> DNA  
 <213> Oryza sativa

<400> 25  
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 cgccgcgcga gcagccggag gacgaaactc gccgtgcgcg cgatggcacc gacgttgctc 180  
 tcgtcgtoga cggcggcggc agctcccccg gggctgaagg agggcatcgc ggggctctac 240  
 gacgagtcgt ccggcgtgtg ggagagcacc tggggcgagc acatgcacca cggcttctac 300  
 gacgccggcg aggccgcctc catgtccgac caccgccgcg cccagatccg catgatcgag 360  
 gaatccctcg ccttcgccgc cgttccccga tgatgcgggt aacaaaccca aaagtgttat 420  
 ttactgtttg gtgttgcaaa tgggggtacc tccaaaaaac tttg 464

<210> 26  
 <211> 128  
 <212> PRT  
 <213> Oryza sativa

<400> 26  
 Ala Arg Val Gln Pro Thr Gly Ala Leu Ala Pro Leu His Pro Leu Leu  
 1 5 10 15  
 Arg Cys Thr Ser Arg His Leu Cys Ala Ser Ala Ser Pro Arg Ala Gly  
 20 25 30  
 Leu Cys Leu His His His Arg Arg Arg Arg Arg Ser Ser Arg Arg Thr  
 35 40 45  
 Lys Leu Ala Val Arg Ala Met Ala Pro Thr Leu Ser Ser Ser Ser Thr  
 50 55 60  
 Ala Ala Ala Ala Pro Pro Gly Leu Lys Glu Gly Ile Ala Gly Leu Tyr  
 65 70 75 80  
 Asp Glu Ser Ser Gly Val Trp Glu Ser Ile Trp Gly Glu His Met His  
 85 90 95  
 His Gly Phe Tyr Asp Ala Gly Glu Ala Ala Ser Met Ser Asp His Arg  
 100 105 110  
 Arg Ala Gln Ile Arg Met Ile Glu Glu Ser Leu Ala Phe Ala Ala Val  
 115 120 125

<210> 27  
 <211> 1189  
 <212> DNA  
 <213> Glycine max

<400> 27  
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 ttcccaatcc cctcgcactt tcgccagaat ccgggtcgga cccaggctcg gggctcctat 120  
 tcgggcatcg gcagcgagct cggagagagg ggagatagta ttggagcaga agccgaagaa 180  
 ggatgacaag aagaagctgc agaagggaaat cgcagagttt tacgacgagt cgtctggctt 240  
 atgggagaaac atttggggcg accacatgca ccatggcttt tatgactcgg attccactgt 300  
 ttcgcttttcg gatcatcgtg ctgctcagat ccgaatgatc caagagtctc ttcgctttgc 360  
 ctctgtttct gaggagcgta gtaaatggcc caagagtata gttgatgttg ggtgtggcat 420  
 aggtggcagc tctagatacc tggccaagaa atttggagca accagtgtag gcatcactct 480  
 gagtcctggt caagctcaaa gagcaaatgc tcttgctgct gctcaaggat tggctgataa 540  
 ggtttccttt caggttgctg acgctctaca gcaaccattc tctgacggcc agtttgatct 600  
 ggtgtggtcc atggagagtg gagagcatat gcctgacaaa gctaagtttg ttggagagtt 660

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agctcgggta gcagcaccag gtgccattat aataatagta acatgggtgcc acaggggatct 720
tggccctgac gaacaatcct tacatccatg ggagcaagat ctcttaaaga agatttgca 780
tgcatattac ctccctgcct ggtgctcaac ttctgattat gttaagttgc tccaatccct 840
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aacgataaaa ggagctttgg ctatgccatt gatgatagag ggatacaaga aagatctaata 1020
taagtttgcc atcattacat gtcgaaaacc tgaataaatg gagaggcagg attactttta 1080
tagaatgaac caagtttcca acaggtcgtt tatttcgata gttgagaaac aagagaaaaa 1140
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<210> 28  
 <211> 350  
 <212> PRT  
 <213> Glycine max

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<400> 28
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                20                      25                     30

Pro Arg Ser Trp Ala Pro Ile Arg Ala Ser Ala Ala Ser Ser Glu Arg
                35                      40                     45

Gly Glu Ile Val Leu Glu Gln Lys Pro Lys Lys Asp Asp Lys Lys Lys
  50                      55                     60

Leu Gln Lys Gly Ile Ala Glu Phe Tyr Asp Glu Ser Ser Gly Leu Trp
  65                      70                     75                     80

Glu Asn Ile Trp Gly Asp His Met His His Gly Phe Tyr Asp Ser Asp
                85                      90                     95

Ser Thr Val Ser Leu Ser Asp His Arg Ala Ala Gln Ile Arg Met Ile
                100                     105                    110

Gln Glu Ser Leu Arg Phe Ala Ser Val Ser Glu Glu Arg Ser Lys Trp
                115                     120                    125

Pro Lys Ser Ile Val Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg
                130                     135                    140

Tyr Leu Ala Lys Lys Phe Gly Ala Thr Ser Val Gly Ile Thr Leu Ser
  145                     150                    155                    160

Pro Val Gln Ala Gln Arg Ala Asn Ala Leu Ala Ala Ala Gln Gly Leu
                165                     170                    175

Ala Asp Lys Val Ser Phe Gln Val Ala Asp Ala Leu Gln Gln Pro Phe
                180                     185                    190

Ser Asp Gly Gln Phe Asp Leu Val Trp Ser Met Glu Ser Gly Glu His
                195                     200                    205

Met Pro Asp Lys Ala Lys Phe Val Gly Glu Leu Ala Arg Val Ala Ala
                210                     215                    220

Pro Gly Ala Ile Ile Ile Ile Val Thr Trp Cys His Arg Asp Leu Gly
  225                     230                    235                    240

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Pro Asp Glu Gln Ser Leu His Pro Trp Glu Gln Asp Leu Leu Lys Lys  
 245 250 255

Ile Cys Asp Ala Tyr Tyr Leu Pro Ala Trp Cys Ser Thr Ser Asp Tyr  
 260 265 270

Val Lys Leu Leu Gln Ser Leu Ser Leu Gln Asp Ile Lys Ser Glu Asp  
 275 280 285

Trp Ser Arg Phe Val Ala Pro Phe Trp Pro Ala Val Ile Arg Ser Ala  
 290 295 300

Phe Thr Trp Lys Gly Leu Ser Ser Leu Leu Ser Ser Gly Gln Lys Thr  
 305 310 315 320

Ile Lys Gly Ala Leu Ala Met Pro Leu Met Ile Glu Gly Tyr Lys Lys  
 325 330 335

Asp Leu Ile Lys Phe Ala Ile Ile Thr Cys Arg Lys Pro Glu  
 340 345 350

<210> 29  
 <211> 1257  
 <212> DNA  
 <213> Triticum aestivum

<220>  
 <221> unsure  
 <222> (31)

<220>  
 <221> unsure  
 <222> (151)

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 ggctgtgctt gccgtctctc ccggccagac ngctccgtgc gcccgatggc gtcgtcgacg 180  
 accgcggccc gggcgacgcg gcgccgccgg ggctgaagga gggcatcgcg gggctctacg 240  
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 agtttgtaag cgagctggca cgcgtcgag ctccaggagc aactatcatc atcgtgacct 720  
 ggtgccatag gaacctcgcg ccgtcggagg actcactgaa acctgacgag ctgaatcttt 780  
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 acaagaaaagg cctcattaag ttcagcatca tcacctgccg caaaccccaa gcagccatag 1080  
 aaggagaacc tgaggccgca tcgcccagtg tagaatagaa cccatgtgat tggaatagac 1140  
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 ctggaagtgg cataggaaag tggttcctaa agcaaaaaaa aaaaaaaaaa aaaaaaa 1257

<210> 30  
 <211> 366

<212> PRT  
 <213> Triticum aestivum

<220>  
 <221> UNSURE  
 <222> (5)

<220>  
 <221> UNSURE  
 <222> (45)

<400> 30  
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 Asp Ala Ala Pro Pro Pro Arg Pro Ser Leu Gly His Ala Ala Arg Pro  
 20 25 30  
 Val Pro Arg Pro Val Leu Pro Leu Leu Pro Ala Arg Xaa Leu Arg Ala  
 35 40 45  
 Pro Asp Gly Val Val Asp Asp Arg Gly Pro Gly Asp Ala Ala Pro Pro  
 50 55 60  
 Gly Leu Lys Glu Gly Ile Ala Gly Leu Tyr Asp Glu Ser Ser Gly Leu  
 65 70 75 80  
 Trp Glu Ser Ile Trp Gly Glu His Met His His Gly Phe Tyr Asp Ser  
 85 90 95  
 Gly Glu Ala Ala Ser Met Ser Asp His Arg Arg Ala Gln Ile Arg Met  
 100 105 110  
 Ile Glu Glu Ala Leu Ala Phe Ala Ala Val Pro Asp Asp Pro Thr Asn  
 115 120 125  
 Lys Pro Lys Thr Ile Val Asp Val Gly Cys Gly Ile Gly Gly Ser Ser  
 130 135 140  
 Arg Tyr Leu Ala Asn Lys Tyr Gly Ala Gln Cys Ser Gly Ile Thr Leu  
 145 150 155 160  
 Ser Pro Val Gln Ala Glu Arg Gly Asn Ala Leu Ala Ala Ala Gln Gly  
 165 170 175  
 Leu Ser Asp Lys Ala Ser Phe Gln Val Ala Asp Ala Leu Glu Gln Pro  
 180 185 190  
 Phe Pro Asp Gly Gln Phe Asp Leu Val Trp Ser Met Glu Ser Gly Glu  
 195 200 205  
 His Met Pro Asn Lys Gln Lys Phe Val Ser Glu Leu Ala Arg Val Ala  
 210 215 220  
 Ala Pro Gly Ala Thr Ile Ile Ile Val Thr Trp Cys His Arg Asn Leu  
 225 230 235 240  
 Ala Pro Ser Glu Asp Ser Leu Lys Pro Asp Glu Leu Asn Leu Leu Lys  
 245 250 255

Lys Ile Cys Asp Ala Tyr Tyr Leu Pro Asp Trp Cys Ser Pro Ser Asp  
 260 265 270  
 Tyr Val Lys Ile Ala Glu Ser Leu Ser Leu Glu Asp Ile Lys Thr Ala  
 275 280 285  
 Asp Trp Ser Glu Asn Val Ala Pro Phe Trp Pro Ala Val Ile Gln Ser  
 290 295 300  
 Ala Leu Thr Trp Lys Gly Leu Thr Ser Leu Leu Arg Ser Gly Trp Lys  
 305 310 315 320  
 Thr Ile Lys Gly Ala Leu Val Met Pro Leu Met Ile Gln Gly Tyr Lys  
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 Lys Gly Leu Ile Lys Phe Ser Ile Ile Thr Cys Arg Lys Pro Gln Ala  
 340 345 350  
 Ala Ile Glu Gly Glu Pro Glu Ala Ala Ser Pro Ser Val Glu  
 355 360 365

<210> 31  
 <211> 1605  
 <212> DNA  
 <213> Catalpa sp.

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 caagcagacg accacttcg ccaccgcccg ggacgggtcc aaagatgcgc atgcagaatt 180  
 caagctgggtg ggcttcaaga atttcgtcag gaccaacccc aagtcggacc acttctgcgt 240  
 ccaccgcttc caccatatag agttctgggtg cggcgacgcc accaacaccg ccaagcgctt 300  
 ctcttggggc ctcggtatgc cctcgtcgc caaatcggat ctttccactg gaaactccgc 360  
 tcatgcctcg tatcttcttc ggtctggcga actcaacttc ctcttcacga gcccttactc 420  
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 gtcttttacc tcttcccatg gcctcgtctg tcgtgcgggtg gctattcagg tcgattcggc 540  
 cttttcggct tactctgcct ccatttcccg cggcgccaaa cccgtgtccg caccgattct 600  
 tttatctgac aacaagactg ccattgcgga ggttcattta tatggagact cagtgttgcg 660  
 attcgtgagc tatggtgata atgggacagg ccagatgga tggttcttgc cgggctttga 720  
 gcctgtggat gatcagatgt cttataaaga attggattat gggattagaa ggctagatca 780  
 tgctgtagga aatgtgcccg aactcgggtc agttgtggat tacttgaaaa aattcacagg 840  
 gtttcatgaa ttgacagagt ttacttcaga ggatgtggga acagcagaaa gtggattgaa 900  
 ttctatggtt ttagcgaaca acaatgaaaa tgtgttggtta cctctgaacg aaccggtgtt 960  
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 caagaacaga gctggagatg tgctgagggg tagcagatg gaggagtgtg agaagttggg 1200  
 gatcttggtg gacagggatg atcaggggac tttgcttcag attttcacca agcctgtggg 1260  
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<210> 32  
 <211> 445  
 <212> PRT  
 <213> Catalpa sp.

<400> 32  
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 20 25 30  
 Thr Asn Pro Lys Ser Asp His Phe Cys Val His Arg Phe His His Ile  
 35 40 45  
 Glu Phe Trp Cys Gly Asp Ala Thr Asn Thr Ala Lys Arg Phe Ser Trp  
 50 55 60  
 Gly Leu Gly Met Pro Leu Val Ala Lys Ser Asp Leu Ser Thr Gly Asn  
 65 70 75 80  
 Ser Ala His Ala Ser Tyr Leu Leu Arg Ser Gly Glu Leu Asn Phe Leu  
 85 90 95  
 Phe Thr Ser Pro Tyr Ser Pro Ser Ile Ser Ala Pro Ser Ser Ala Ala  
 100 105 110  
 Ile Pro Ser Phe Ser Phe Ser Thr Tyr Gln Ser Phe Thr Ser Ser His  
 115 120 125  
 Gly Leu Ala Val Arg Ala Val Ala Ile Gln Val Asp Ser Ala Phe Ser  
 130 135 140  
 Ala Tyr Ser Ala Ser Ile Ser Arg Gly Ala Lys Pro Val Ser Ala Pro  
 145 150 155 160  
 Ile Leu Leu Ser Asp Asn Lys Thr Ala Ile Ala Glu Val His Leu Tyr  
 165 170 175  
 Gly Asp Ser Val Leu Arg Phe Val Ser Tyr Gly Asp Asn Gly Thr Gly  
 180 185 190  
 Pro Asp Gly Trp Phe Leu Pro Gly Phe Glu Pro Val Asp Asp Gln Met  
 195 200 205  
 Ser Tyr Lys Glu Leu Asp Tyr Gly Ile Arg Arg Leu Asp His Ala Val  
 210 215 220  
 Gly Asn Val Pro Glu Leu Gly Pro Val Val Asp Tyr Leu Lys Lys Phe  
 225 230 235 240  
 Thr Gly Phe His Glu Phe Ala Glu Phe Thr Ser Glu Asp Val Gly Thr  
 245 250 255  
 Ala Glu Ser Gly Leu Asn Ser Met Val Leu Ala Asn Asn Asn Glu Asn  
 260 265 270  
 Val Leu Leu Pro Leu Asn Glu Pro Val Phe Gly Thr Lys Arg Lys Ser  
 275 280 285  
 Gln Ile Gln Thr Tyr Leu Glu His Asn Glu Gly Pro Gly Val Gln His  
 290 295 300  
 Leu Ala Leu Val Ser Glu Asp Ile Phe Asn Thr Leu Arg Glu Met Arg  
 305 310 315 320

Lys Arg Ser Gly Val Gly Gly Phe Glu Phe Met Pro Ser Pro Pro Leu  
 325 330 335  
 Thr Tyr Tyr Lys Asn Leu Lys Asn Arg Ala Gly Asp Val Leu Arg Asp  
 340 345 350  
 Glu Gln Ile Glu Glu Cys Glu Lys Leu Gly Ile Leu Val Asp Arg Asp  
 355 360 365  
 Asp Gln Gly Thr Leu Leu Gln Ile Phe Thr Lys Pro Val Gly Asp Arg  
 370 375 380  
 Pro Thr Leu Phe Ile Glu Ile Ile Gln Arg Ile Gly Cys Met Leu Lys  
 385 390 395 400  
 Asp Glu Gln Gly Lys Leu Tyr Gln Lys Ser Gly Cys Gly Gly Phe Gly  
 405 410 415  
 Lys Gly Asn Phe Ser Glu Leu Phe Lys Ser Ile Glu Glu Tyr Glu Lys  
 420 425 430  
 Met Leu Glu Ala Lys Gln Val Thr Glu Thr Ala Ser Ala  
 435 440 445

<210> 33  
 <211> 1106  
 <212> DNA  
 <213> Oryza sativa

<400> 33  
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 tcgctccggt agccgcgtac atctccgggt tcaccgggtt ccacgagttc gccgagttca 120  
 ccgccgagga cgtgggcacc gccgagagcg gcctcaactc ggtgggtgctc gccacaacacg 180  
 cggagaccgt gctgctgccc ctcaacgagc cgggtgcacgg caccaagcgg cggagccaga 240  
 tacagacgta cctggaccac cacggcggcc cgggggtgca gcacatcgcg ctggccagcg 300  
 acgacgtgct cgggacgctg agggagatgc gggcgcgctc cgccatgggc ggcttcgagt 360  
 tcttggcgcc gccgcgcc aactactacg acggcgtgcg ggggcgcgcc ggggacgtgc 420  
 tctcggagga gcagatcaac gaggccagc agctcggggt gctcgtggac agggatgacc 480  
 aggggggtgt gctccagatc ttcaccaagc cagtaggaga caggccaacc ttttcttg 540  
 agatgataca aaggattggg tgcattggag aggatgagag tgggcaggag taccagaagg 600  
 gcggctgcgg cgggtttggg aagggaact tctcggagct gttcaagtcc attgaggagt 660  
 atgagaaatc ccttgaagcc aagcaagccc ctacagttca aggatcctag gtaggaactg 720  
 gaggcctgga gcaacagatg taaccagtgt atttgtatta tggagcagaa gaaaaaagat 780  
 gtgctttcac tgctttgtga tatgtgtcat gcaagttgat gttgtaattt gtggaagctg 840  
 aagacaaatg atggtacaat cactgtaata gataatagac atggatcaca tacaagaatg 900  
 taacctagtg ttggcattgc tgctgtacaa tcttgcttgg aaataaaata ataatacaacc 960  
 tggagaaaga atgtaacctc ctgttggcat tgctgatgta caatcttttt ttggaaataa 1020  
 aataagaatc cccccaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1080  
 aaaaaaaaaa aaaaaaaaaa aaaaaa 1106

<210> 34  
 <211> 235  
 <212> PRT  
 <213> Oryza sativa

<400> 34  
 Thr Arg Lys Ser Tyr Gly Leu Arg Arg Phe Asp His Val Val Gly Asn  
 1 5 10 15

Val Pro Glu Leu Ala Pro Val Ala Ala Tyr Ile Ser Gly Phe Thr Gly  
                     20                    25                    30  
 Phe His Glu Phe Ala Glu Phe Thr Ala Glu Asp Val Gly Thr Ala Glu  
                     35                    40                    45  
 Ser Gly Leu Asn Ser Val Val Leu Ala Asn Asn Ala Glu Thr Val Leu  
                     50                    55                    60  
 Leu Pro Leu Asn Glu Pro Val His Gly Thr Lys Arg Arg Ser Gln Ile  
                     65                    70                    75                    80  
 Gln Thr Tyr Leu Asp His His Gly Gly Pro Gly Val Gln His Ile Ala  
                     85                    90                    95  
 Leu Ala Ser Asp Asp Val Leu Gly Thr Leu Arg Glu Met Arg Ala Arg  
                     100                    105                    110  
 Ser Ala Met Gly Gly Phe Glu Phe Leu Ala Pro Pro Pro Pro Asn Tyr  
                     115                    120                    125  
 Tyr Asp Gly Val Arg Arg Arg Ala Gly Asp Val Leu Ser Glu Glu Gln  
                     130                    135                    140  
 Ile Asn Glu Cys Gln Glu Leu Gly Val Leu Val Asp Arg Asp Asp Gln  
                     145                    150                    155                    160  
 Gly Val Leu Leu Gln Ile Phe Thr Lys Pro Val Gly Asp Arg Pro Thr  
                     165                    170                    175  
 Phe Phe Leu Glu Met Ile Gln Arg Ile Gly Cys Met Glu Lys Asp Glu  
                     180                    185                    190  
 Ser Gly Gln Glu Tyr Gln Lys Gly Gly Cys Gly Gly Phe Gly Lys Gly  
                     195                    200                    205  
 Asn Phe Ser Glu Leu Phe Lys Ser Ile Glu Glu Tyr Glu Lys Ser Leu  
                     210                    215                    220  
 Glu Ala Lys Gln Ala Pro Thr Val Gln Gly Ser  
                     225                    230                    235

<210> 35  
 <211> 1550  
 <212> DNA  
 <213> Glycine max

<400> 35  
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 agcccaagcc caacctgggt ttaagctcgt cggtttcaaa aacttcgtcc gaaccaatcc 120  
 taagtccgac cgctttcaag tcaaccgctt ccaccacatc gagttctggt gcaccgatgc 180  
 caccaacgcc tctcgccgat tctcttgggg acttggaaatg cctattgtgg caaaatctga 240  
 tctctccacc ggaaaccaa tccacgcctc ctacctctc cgctccggcg acctctcctt 300  
 cctctttctcc gctccttact ctccctctct ctccgcgggc tctccgctg cctcctccgc 360  
 ctccattccc agtttcgacg ccgccacctg ccttgccctt gctgccaaac acggcttcgg 420  
 cgtcgcgcgc atcgccttgg aagtcgccga cgcggaagcc gctttcagcg ccagcgtcgc 480  
 gaaaggagcc gagccggcgt cgccgcgggt tctcgtcgac gatcgcaacc gttcgcgga 540  
 ggtgcgcctc tacggcgacg tgggtgctcg ctacgtcagc tacaaggacg ccgcgcgcga 600  
 ggcgccacac gcagatccgt cgcggtggtt cctgccggga ttcgaggccg cggcgtcgtc 660  
 gtcttcggtt ccggagctgg actacgggat ccggcggtcg gaccacgccg tcgggaacgt 720

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tccggagctg ggcgcggcgg tgaggtaacct gaaaggcttc agcggattcc acgagttcgc 780
ggagttcacc gcggaggacg tgggaacgag cgagagcggg ttgaactcgg tggttctggc 840
gaacaactcg gagacggtgt tgctgccgct gaacgagccg gtttacggaa cgaagaggaa 900
gagccagatt gagacgtatt tggaaacaaa cgaagggtgct ggtgtgcagc accttgcgct 960
tgttactcac gacatcttca ccacactgag agagatgaga aagcgaagtt tccttggtgg 1020
at ttgagttc atgccttctc ctccctccac ctattacgcc aacctccaca accgtgccgc 1080
tgatgtgttg accgttgacc agattaagca gtgtgaggag cttgggattc ttgttgacag 1140
agatgatcag ggcactctgc ttcagatttt caccaagcct gttggggaca ggccaacgat 1200
attcatagag ataattcaga ggatcgggtg catggtggag gatgaggaag ggaaggtgta 1260
ccagaagggt gcatgtgggg gttttgggaa aggaatttt tctgagcttt tcaaattccat 1320
tgaagaatat gagaagactt tggaagctaa aagaaccgcg taagcacatt ggaagaacac 1380
aaataactcct ttgttgaaat gattaatgag gaatcaatgt ggcatagggt gtttatactc 1440
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cttttatgga tagtattttt ctattaaaaa aaaaaaaaaa aaaaaaaaaa 1550

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<210> 36  
 <211> 449  
 <212> PRT  
 <213> Glycine max

<400> 36

Met Pro Ile Pro Met Cys Asn Glu Ile Gln Ala Gln Ala Gln Ala Gln  
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Ala Gln Ala Gln Pro Gly Phe Lys Leu Val Gly Phe Lys Asn Phe Val  
 20 25 30

Arg Thr Asn Pro Lys Ser Asp Arg Phe Gln Val Asn Arg Phe His His  
 35 40 45

Ile Glu Phe Trp Cys Thr Asp Ala Thr Asn Ala Ser Arg Arg Phe Ser  
 50 55 60

Trp Gly Leu Gly Met Pro Ile Val Ala Lys Ser Asp Leu Ser Thr Gly  
 65 70 75 80

Asn Gln Ile His Ala Ser Tyr Leu Leu Arg Ser Gly Asp Leu Ser Phe  
 85 90 95

Leu Phe Ser Ala Pro Tyr Ser Pro Ser Leu Ser Ala Gly Ser Ser Ala  
 100 105 110

Ala Ser Ser Ala Ser Ile Pro Ser Phe Asp Ala Ala Thr Cys Leu Ala  
 115 120 125

Phe Ala Ala Lys His Gly Phe Gly Val Arg Ala Ile Ala Leu Glu Val  
 130 135 140

Ala Asp Ala Glu Ala Ala Phe Ser Ala Ser Val Ala Lys Gly Ala Glu  
 145 150 155 160

Pro Ala Ser Pro Pro Val Leu Val Asp Asp Arg Thr Gly Phe Ala Glu  
 165 170 175

Val Arg Leu Tyr Gly Asp Val Val Leu Arg Tyr Val Ser Tyr Lys Asp  
 180 185 190

Ala Ala Pro Gln Ala Pro His Ala Asp Pro Ser Arg Trp Phe Leu Pro  
 195 200 205

Gly Phe Glu Ala Ala Ala Ser Ser Ser Ser Phe Pro Glu Leu Asp Tyr  
 210 215 220  
 Gly Ile Arg Arg Leu Asp His Ala Val Gly Asn Val Pro Glu Leu Ala  
 225 230 235 240  
 Pro Ala Val Arg Tyr Leu Lys Gly Phe Ser Gly Phe His Glu Phe Ala  
 245 250 255  
 Glu Phe Thr Ala Glu Asp Val Gly Thr Ser Glu Ser Gly Leu Asn Ser  
 260 265 270  
 Val Val Leu Ala Asn Asn Ser Glu Thr Val Leu Leu Pro Leu Asn Glu  
 275 280 285  
 Pro Val Tyr Gly Thr Lys Arg Lys Ser Gln Ile Glu Thr Tyr Leu Glu  
 290 295 300  
 His Asn Glu Gly Ala Gly Val Gln His Leu Ala Leu Val Thr His Asp  
 305 310 315 320  
 Ile Phe Thr Thr Leu Arg Glu Met Arg Lys Arg Ser Phe Leu Gly Gly  
 325 330 335  
 Phe Glu Phe Met Pro Ser Pro Pro Pro Thr Tyr Tyr Ala Asn Leu His  
 340 345 350  
 Asn Arg Ala Ala Asp Val Leu Thr Val Asp Gln Ile Lys Gln Cys Glu  
 355 360 365  
 Glu Leu Gly Ile Leu Val Asp Arg Asp Asp Gln Gly Thr Leu Leu Gln  
 370 375 380  
 Ile Phe Thr Lys Pro Val Gly Asp Arg Pro Thr Ile Phe Ile Glu Ile  
 385 390 395 400  
 Ile Gln Arg Ile Gly Cys Met Val Glu Asp Glu Glu Gly Lys Val Tyr  
 405 410 415  
 Gln Lys Gly Ala Cys Gly Gly Phe Gly Lys Gly Asn Phe Ser Glu Leu  
 420 425 430  
 Phe Lys Ser Ile Glu Glu Tyr Glu Lys Thr Leu Glu Ala Lys Arg Thr  
 435 440 445

Ala

<210> 37  
 <211> 1614  
 <212> DNA  
 <213> Triticum aestivum

<400> 37  
 gcacgagcaa gaagcgaaca cacaccatgc cgcccacccc caccaccccc gcagccaccg 60  
 gcgcgcgcgc ggtgacgcgc gagcacgcgc ggccgcgcgc aatggtccgc ttcaaccgcg 120  
 gcagcgaccg cttccacacg ctgcgccttc accacgtcga gttctggtgc gcggacgcgc 180  
 cctccgcgcg cggecgcttc gccttcgcgc tcggcgcgcc gctcgccgcc aggtccgacc 240  
 tctccacggg gaactccgtg cagcgcctcc agctgctccg ctcgggcaac ctgcgccttc 300  
 tcttcacggc cccctacgcc aacggctgcg acgccgccac cgcctccctg ccctccttct 360

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ccgccgacgc cgcgcgccag ttctccgcgg accacggcct cgcggtgcgc tccatagcgc 420
tgcgcgtcgc ygacgctgcc gaggccttcc gcgccagcgt cgacgggggc gcgcgcccg 480
ccttcagccc tgtggacctc ggccgcggct tcggcttcgc ggaggtcgag ctctacggcg 540
acgtcgtgct ccgcttcgtc agccaccggg acggcaggga cgtgcccttc ttgccggggt 600
tcgagggcgt gagcaacca gacgcctggg actacggcct gacgcggttc gaccacgtcg 660
tcggcaacgt cccggagctt gccccgcgc cggcctacgt cgccgggttc acgggggttc 720
acgagttcgc cgagttcacg acggaggacg tgggcacggc cgagagcggg ctcaactcga 780
tggtgctcgc caacaactcg gagggcgtgc tgctgccgct caacgagccg gtgcacggca 840
ccaagcgccg gagccagata cagacgttcc tggaacacca cggcggtcgc ggcggtcgcg 900
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tcgtcgacag ggacgaccaa ggggtgttgc tacaatctt caccaagcca gtaggggaca 1140
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gggaagagta ccagaagggt ggctgcggcg ggctcggcaa aggcaacttc tccgagctgt 1260
tcaagtccat tgaagattac gagaagtccc ttgaagccaa gcaatctgct gcagttcagg 1320
gatcatagga tagaagctgg agctggagga gctgatccag tactttgtat caggtctcat 1380
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ggctcggctc acacatgaac aaaatgtact gttggcattg ttgtataatc ttgcttgcaa 1560
gtaaaaataa gaagaaccga ttttgagttc tgcataaaaa aaaaaaaaaa aaaa 1614

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&lt;210&gt; 38

&lt;211&gt; 433

&lt;212&gt; PRT

&lt;213&gt; Triticum aestivum

&lt;400&gt; 38

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Met Pro Pro Thr Pro Thr Thr Pro Ala Ala Thr Gly Ala Ala Ala Val
 1             5             10             15

Thr Pro Glu His Ala Arg Pro Arg Arg Met Val Arg Phe Asn Pro Arg
          20             25             30

Ser Asp Arg Phe His Thr Leu Ala Phe His His Val Glu Phe Trp Cys
          35             40             45

Ala Asp Ala Ala Ser Ala Ala Gly Arg Phe Ala Phe Ala Leu Gly Ala
 50             55             60

Pro Leu Ala Ala Arg Ser Asp Leu Ser Thr Gly Asn Ser Val His Ala
 65             70             75             80

Ser Gln Leu Leu Arg Ser Gly Asn Leu Ala Phe Leu Phe Thr Ala Pro
          85             90             95

Tyr Ala Asn Gly Cys Asp Ala Ala Thr Ala Ser Leu Pro Ser Phe Ser
          100             105             110

Ala Asp Ala Ala Arg Gln Phe Ser Ala Asp His Gly Leu Ala Val Arg
          115             120             125

Ser Ile Ala Leu Arg Val Ala Asp Ala Ala Glu Ala Phe Arg Ala Ser
          130             135             140

Val Asp Gly Gly Ala Arg Pro Ala Phe Ser Pro Val Asp Leu Gly Arg
          145             150             155             160

Gly Phe Gly Phe Ala Glu Val Glu Leu Tyr Gly Asp Val Val Leu Arg
          165             170             175

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Phe Val Ser His Pro Asp Gly Arg Asp Val Pro Phe Leu Pro Gly Phe  
 180 185 190  
 Glu Gly Val Ser Asn Pro Asp Ala Val Asp Tyr Gly Leu Thr Arg Phe  
 195 200 205  
 Asp His Val Val Gly Asn Val Pro Glu Leu Ala Pro Ala Ala Ala Tyr  
 210 215 220  
 Val Ala Gly Phe Thr Gly Phe His Glu Phe Ala Glu Phe Thr Thr Glu  
 225 230 235 240  
 Asp Val Gly Thr Ala Glu Ser Gly Leu Asn Ser Met Val Leu Ala Asn  
 245 250 255  
 Asn Ser Glu Gly Val Leu Leu Pro Leu Asn Glu Pro Val His Gly Thr  
 260 265 270  
 Lys Arg Arg Ser Gln Ile Gln Thr Phe Leu Glu His His Gly Gly Ser  
 275 280 285  
 Gly Val Gln His Ile Ala Val Ala Ser Ser Asp Val Leu Arg Thr Leu  
 290 295 300  
 Arg Glu Met Arg Ala Arg Ser Ala Met Gly Gly Phe Asp Phe Leu Pro  
 305 310 315 320  
 Pro Pro Leu Pro Lys Tyr Tyr Glu Gly Val Arg Arg Ile Ala Gly Asp  
 325 330 335  
 Val Leu Ser Glu Ala Gln Ile Lys Glu Cys Gln Glu Leu Gly Val Leu  
 340 345 350  
 Val Asp Arg Asp Asp Gln Gly Val Leu Leu Gln Ile Phe Thr Lys Pro  
 355 360 365  
 Val Gly Asp Arg Pro Thr Leu Phe Leu Glu Met Ile Gln Arg Ile Gly  
 370 375 380  
 Cys Met Glu Lys Asp Glu Arg Gly Glu Glu Tyr Gln Lys Gly Gly Cys  
 385 390 395 400  
 Gly Gly Phe Gly Lys Gly Asn Phe Ser Glu Leu Phe Lys Ser Ile Glu  
 405 410 415  
 Asp Tyr Glu Lys Ser Leu Glu Ala Lys Gln Ser Ala Ala Val Gln Gly  
 420 425 430

Ser

<210> 39  
 <211> 317  
 <212> PRT  
 <213> Synechocystis sp.

<400> 39  
 Met Val Tyr His Val Arg Pro Lys His Ala Leu Phe Leu Ala Phe Tyr  
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Cys Tyr Phe Ser Leu Leu Thr Met Ala Ser Ala Thr Ile Ala Ser Ala  
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 Asp Leu Tyr Glu Lys Ile Lys Asn Phe Tyr Asp Asp Ser Ser Gly Leu  
                   35                  40                  45  
 Trp Glu Asp Val Trp Gly Glu His Met His His Gly Tyr Tyr Gly Pro  
                   50                  55                  60  
 His Gly Thr Tyr Arg Ile Asp Arg Arg Gln Ala Gln Ile Asp Leu Ile  
                   65                  70                  75                  80  
 Lys Glu Leu Leu Ala Trp Ala Val Pro Gln Asn Ser Ala Lys Pro Arg  
                   85                  90                  95  
 Lys Ile Leu Asp Leu Gly Cys Gly Ile Gly Gly Ser Ser Leu Tyr Leu  
                   100                  105                  110  
 Ala Gln Gln His Gln Ala Glu Val Met Gly Ala Ser Leu Ser Pro Val  
                   115                  120                  125  
 Gln Val Glu Arg Ala Gly Glu Arg Ala Arg Ala Leu Gly Leu Gly Ser  
                   130                  135                  140  
 Thr Cys Gln Phe Gln Val Ala Asn Ala Leu Asp Leu Pro Phe Ala Ser  
                   145                  150                  155                  160  
 Asp Ser Phe Asp Trp Val Trp Ser Leu Glu Ser Gly Glu His Met Pro  
                   165                  170                  175  
 Asn Lys Ala Gln Phe Leu Gln Glu Ala Trp Arg Val Leu Lys Pro Gly  
                   180                  185                  190  
 Gly Arg Leu Ile Leu Ala Thr Trp Cys His Arg Pro Ile Asp Pro Gly  
                   195                  200                  205  
 Asn Gly Pro Leu Thr Ala Asp Glu Arg Arg His Leu Gln Ala Ile Tyr  
                   210                  215                  220  
 Asp Val Tyr Cys Leu Pro Tyr Val Val Ser Leu Pro Asp Tyr Glu Ala  
                   225                  230                  235                  240  
 Ile Ala Arg Glu Cys Gly Phe Gly Glu Ile Lys Thr Ala Asp Trp Ser  
                   245                  250                  255  
 Val Ala Val Ala Pro Phe Trp Asp Arg Val Ile Glu Ser Ala Phe Asp  
                   260                  265                  270  
 Pro Arg Val Leu Trp Ala Leu Gly Gln Ala Gly Pro Lys Ile Ile Asn  
                   275                  280                  285  
 Ala Ala Leu Cys Leu Arg Leu Met Lys Trp Gly Tyr Glu Arg Gly Leu  
                   290                  295                  300  
 Val Arg Phe Gly Leu Leu Thr Gly Ile Lys Pro Leu Val  
                   305                  310                  315  
 <210> 40  
 <211> 348

&lt;212&gt; PRT

&lt;213&gt; Arabidopsis thaliana

&lt;400&gt; 40

Met Lys Ala Thr Leu Ala Ala Pro Ser Ser Leu Thr Ser Leu Pro Tyr  
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Arg Thr Asn Ser Ser Phe Gly Ser Lys Ser Ser Leu Leu Phe Arg Ser  
 20 25 30

Pro Ser Ser Ser Ser Ser Val Ser Met Thr Thr Thr Arg Gly Asn Val  
 35 40 45

Ala Val Ala Ala Ala Ala Thr Ser Thr Glu Ala Leu Arg Lys Gly Ile  
 50 55 60

Ala Glu Phe Tyr Asn Glu Thr Ser Gly Leu Trp Glu Glu Ile Trp Gly  
 65 70 75 80

Asp His Met His His Gly Phe Tyr Asp Pro Asp Ser Ser Val Gln Leu  
 85 90 95

Ser Asp Ser Gly His Lys Glu Ala Gln Ile Arg Met Ile Glu Glu Ser  
 100 105 110

Leu Arg Phe Ala Gly Val Thr Asp Glu Glu Glu Glu Lys Lys Ile Lys  
 115 120 125

Lys Val Val Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg Tyr Leu  
 130 135 140

Ala Ser Lys Phe Gly Ala Glu Cys Ile Gly Ile Thr Leu Ser Pro Val  
 145 150 155 160

Gln Ala Lys Arg Ala Asn Asp Leu Ala Ala Ala Gln Ser Leu Ser His  
 165 170 175

Lys Ala Ser Phe Gln Val Ala Asp Ala Leu Asp Gln Pro Phe Glu Asp  
 180 185 190

Gly Lys Phe Asp Leu Val Trp Ser Met Glu Ser Gly Glu His Met Pro  
 195 200 205

Asp Lys Ala Lys Phe Val Lys Glu Leu Val Arg Val Ala Ala Pro Gly  
 210 215 220

Gly Arg Ile Ile Ile Val Thr Trp Cys His Arg Asn Leu Ser Ala Gly  
 225 230 235 240

Glu Glu Ala Leu Gln Pro Trp Glu Gln Asn Ile Leu Asp Lys Ile Cys  
 245 250 255

Lys Thr Phe Tyr Leu Pro Ala Trp Cys Ser Thr Asp Asp Tyr Val Asn  
 260 265 270

Leu Leu Gln Ser His Ser Leu Gln Asp Ile Lys Cys Ala Asp Trp Ser  
 275 280 285

Glu Asn Val Ala Pro Phe Trp Pro Ala Val Ile Arg Thr Ala Leu Thr  
 290 295 300

Trp Lys Gly Leu Val Ser Leu Leu Arg Ser Gly Met Lys Ser Ile Lys  
 305 310 315 320

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<213> Hordeum vulgare

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<212> PRT

<213> Arabidopsis thaliana

<400> 43

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420 425 430

Glu Tyr Glu Lys Thr Leu Glu Ala Lys Gln Leu Val Gly  
435 440 445

BNSDOCID: <WO 0032757A3 | >

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# INTERNATIONAL SEARCH REPORT

Internat : Application No  
PCT/US 99/28588

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N9/10 C12N9/02 C12N15/82 C12N15/29 C12N15/63  
C12N15/83 C12Q1/48 C12Q1/26 C12Q1/68 G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE EMBL [Online] Accession No. D64004, 4 October 1995 (1995-10-04) "Synechocystis sp. PCC6803 complete genome, delta(24)-sterol C-methyltransferase" XP002135563 see p. 22, l. 38 of present description: 1001725 corresponds to BAA10562 and D64004 the whole document</p> <p style="text-align: center;">--- -/--</p>	1,3-11, 13-25

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### ° Special categories of cited documents :

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- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*Z\* document member of the same patent family

Date of the actual completion of the international search

12 April 2000

Date of mailing of the international search report

01 09 00

Name and mailing address of the ISA

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Authorized officer

Herrmann, K

## INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 99/28588

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BARTLEY, G.E. ET AL.: "Cloning of an Arabidopsis thaliana cDNA for p-hydroxyphenylpyruvate dioxygenase (Accession No. U89267)" PLANT PHYSIOL., vol. 113, 1997, pages 1465-1465, XP002135561 the whole document	18
A	& DATABASE EMBL [Online] Accession No. U89267, 19 March 1997 (1997-03-19) "Arabidopsis thaliana p-hydroxyphenylpyruvate dioxygenase (HPD) mRNA, complete cds" the whole document ---	
A	SHINTANI, D. AND DELLAPENNA, D.: "A new paradigm for plant biochemistry: Nutritional Genomics. Vitamin E synthesis and human nutrition as an example.", Abstract of the oral presentation at the annual meeting of The American Society of Plant Physiology, 28.06.1998 XP002135562 the whole document ---	
P,X	SHINTANI, D. AND DELLAPENNA, D.: "Elevating the vitamin E content of plants through metabolic engineering" SCIENCE, vol. 282, no. 5396, 11 December 1998 (1998-12-11), pages 2098-2100, XP000887122 the whole document ---	
P,A	WO 99 04622 A (UNIV NEVADA) 4 February 1999 (1999-02-04) -----	

# INTERNATIONAL SEARCH REPORT

Inter. l. application No.  
PCT/US 99/28588

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
  
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1, 3-11, 13-25 (all partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

1. Claims: Invention 1: Claims 1, 3-11, 13-25 (all partially)

Corn gamma-tocopherol methyltransferase:

Polynucleotide encoding a polypeptide as in SEQ ID NO: 2 or 22, polypeptide as in SEQ ID NO:2 or 22, polynucleotide comprising a nucleotide sequence as in SEQ ID NO:1 or 21. Subject-matter relating to corn gamma-tocopherol methyltransferase.

2. Claims: Invention 2: Claims 1, 3-11, 13-25 (all partially)

Rice gamma-tocopherol methyltransferase:

Polynucleotide encoding a polypeptide as in SEQ ID NO:4, 6, 24 or 26, polypeptide as in SEQ ID NO:4, 6, 24 or 26, polynucleotide comprising a nucleotide sequence as in SEQ ID NO:3, 5, 23 or 25. Subject-matter relating to rice gamma-tocopherol methyltransferase.

3. Claims: Invention 3: Claims 1, 3-11, 13-25 (all partially)

Soybean gamma-tocopherol methyltransferase:

Polynucleotide encoding a polypeptide as in SEQ ID NO:8 or 28, polypeptide as in SEQ ID NO:8 or 28, polynucleotide comprising a nucleotide sequence as in SEQ ID NO:7 or 27. Subject-matter relating to soybean gamma-tocopherol methyltransferase.

4. Claims: Invention 4: Claims 1, 3-11, 13-25 (all partially)

Wheat gamma-tocopherol methyltransferase:

Polynucleotide encoding a polypeptide as in SEQ ID NO:8 or 28, polypeptide as in SEQ ID NO:10 or 30, polynucleotide comprising a nucleotide sequence as in SEQ ID NO:10 or 30. Subject-matter relating to wheat gamma-tocopherol methyltransferase.

5. Claims: Invention 5: Claims 2-10, 12-19, 21-25 (all partially)

Rice 4-hydroxyphenylpyruvate dioxygenase:

Polynucleotide encoding a polypeptide as in SEQ ID NO:12, 14 or 34, polypeptide as in SEQ ID NO:12, 14 or 34, polynucleotide comprising a nucleotide sequence as in SEQ ID NO:11, 13 or 33. Subject-matter relating to rice 4-hydroxyphenylpyruvate dioxygenase.

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

6. Claims: Invention 6: Claims 2-10, 12-19,  
21-25 (all partially)

**Soybean 4-hydroxyphenylpyruvate dioxygenase:**

Polynucleotide encoding a polypeptide as in SEQ ID NO:16 or 36, polypeptide as in SEQ ID NO:16 or 36, polynucleotide comprising a nucleotide sequence as in SEQ ID NO:15 or 35. Subject-matter relating to soybean 4-hydroxyphenylpyruvate dioxygenase.

7. Claims: Invention 7: Claims 2-10, 12-19,  
21-25 (all partially)

**Veronica 4-hydroxyphenylpyruvate dioxygenase:**

Polynucleotide encoding a polypeptide as in SEQ ID NO:18, polypeptide as in SEQ ID NO:18, polynucleotide comprising a nucleotide sequence as in SEQ ID NO:17. Subject-matter relating to veronica 4-hydroxyphenylpyruvate dioxygenase.

8. Claims: Invention 8: Claims 2-10, 12-19,  
21-25 (all partially)

**Wheat 4-hydroxyphenylpyruvate dioxygenase:**

Polynucleotide encoding a polypeptide as in SEQ ID NO:20 or 38, polypeptide as in SEQ ID NO:20 or 38, polynucleotide comprising a nucleotide sequence as in SEQ ID NO:19 or 37. Subject-matter relating to wheat 4-hydroxyphenylpyruvate dioxygenase.

9. Claims: Invention 9: Claims 2-10, 12-19,  
21-25 (all partially)

**Catalpa 4-hydroxyphenylpyruvate dioxygenase:**

Polynucleotide encoding a polypeptide as in SEQ ID NO:32, polypeptide as in SEQ ID NO:32, polynucleotide comprising a nucleotide sequence as in SEQ ID NO:31. Subject-matter relating to catalpa 4-hydroxyphenylpyruvate dioxygenase.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/28588

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9904622 A.	04-02-1999	AU 8506198 A EP 1009812 A	16-02-1999 21-06-2000
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